

DOCKET NO: 286808US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
FRANK MATTNER, ET AL.	EXAMINER: KOLKER
SERIAL NO: 10/571,469	:
FILED: MARCH 13, 2006	ART UNIT: 1649
FOR: APHERESIS DEVICE	:

DECLARATION UNDER 37 CFR 1.132

COMMISSIONER FOR PATENTS  
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Eliezer Masliah, M.D., state:

1. I have substantial experience and expertise in the molecular mechanisms of synaptic pathology in neurodegenerative diseases, especially Alzheimer's Disease (AD), as well as in the development of new experimental therapies for AD. I have been working on this topics concerning AD for more than 20years. (Please see the attached *Curriculum Vitae and Biographical Sketch*).

2. I have conducted research collaboration with the assignees of the above-identified application and I do not have any interest in the outcome of this application. I was not, or am currently not, or will not be compensated monetarily for my time to provide this Declaration.

3. I am familiar with the application and current claims that are pending in the above-identified application in that the claims define a method for treating Alzheimer's Disease (AD) in a patient by contacting blood or plasma flow of a patient suffering from AD

or a patient with a risk for AD with an apheresis device, the apheresis device including a solid carrier, said solid carrier having anti-amyloid- $\beta$  precursor protein (APP) antibodies attached to a surface of the solid carrier so as to reduce APP polypeptides in the brain and treat AD in the patient.

4. I also understand that the United States Patent and Trademark Office (USPTO) has rejected this method as something that would have been obvious to do to a person in this field based on the publications of DeMattos (2001) (*Proc Natl Acad Sci, USA* 98:8850-8855) and Kojima (2001) (*J Biochem Biophys Methods* 49:241-251).

5. I do not agree with the conclusions of the USPTO and one in this field would not have thought to do what is defined in the claims of this application based on the publications of DeMattos and Kojima.

6. The USPTO misinterpreted some of the data and results of the DeMattos paper and did not take into consideration the difference between an antibody which is administered to a patient and acts intra-corporally and an antibody which acts via immobilization on the solid surface extra-corporally as in the claims of this application.

7. First, the USPTO refers to a "peripheral" administration of m266 and from this I can only infer that the USPTO views that m266 antibody exclusively acts from the periphery (the blood) and does not even occur in the brain or in the CSF. However, this is not true. Even in the DeMattos et al, it is clear that m266 also crosses the blood brain barrier (BBB); the separation of circulating blood and cerebrospinal fluid (CSF) in the central nervous system (CNS); occurring along all capillaries and consisting of tight junctions around the capillaries that do not exist in normal circulation. DeMattos stated on page 8852, right column, that the concentration of the antibody in CSF was  $12.0 \pm 0.95$  ng/ml. Despite the fact that in DeMattos it was speculated that this amount "should only be able to account for an increase of A $\beta$  of less than 1 ng/ml", the DeMattos statement turned out to be rather due to a

detectability problem because in a follow-up publication (see enclosed article of Dodart et al. (Nature Neuroscience 5 (5) (2002): 452- 457) the same group found that the presence of the m266 antibody in the brain was important for reversing memory deficits in an Alzheimer's disease model. It is further shown that m266 can be detected in the brain in the high-dose groups whereas plasma increase is detected in all groups. Fig. 4D of Dodart et al. again showed that there is direct evidence for the presence of m266 in the brain. This is also confirmed in the second paragraph on page 455, left column of Dodart et al., where it is stated that the antibody/A $\beta$  complex was also found in CSF at the lowest dose found to be effective in reversing learning and memory impairment. This again shows that besides the detectability problem this group had -the failure to detect m266 does not mean that there is no antibody present) - one would see the action of a peripherally administered anti A $\beta$  antibody such as m266 (or of two additional examples: m3D6 and m10D5 also mentioned in DeMattos, left column, third paragraph, last sentence) as being able to cross the BBB and have a direct effect on A $\beta$ /A $\beta$  deposits in the brain.

8. It is therefore clear that, in contrast to the interpretation of the USPTO, administration of the m266 antibody is not connected to an exclusively peripheral effect but that this antibody is present in the brain and its extra-cellular compartment (in the CSF) *in vivo* following peripheral administration. Indeed, other antibodies for which crossing of the BBB is known [see Kotilinek et al (J. Neuroscience 22 (2002), 6331-6335), Bard et al. (Nat. Med. 6 (2000), 916-.919),,and, Hokoshi et al. (BBRC 325 (2004), 384-387] exert effects in the brain.

9. It is also known that an antibody in the CNS can foster the release of A $\beta$  from the CNS significantly (see DeMattos., page 8852; and the attached Kotilinek et al, Bard et al, Hokoshi et al and Wilcox et al. (J. Neuroscience 23 (2003), 3745-3751) showing the crossing of peripherally administered anti-A $\beta$ -antibodies over the BBB and the presence and

functionality of such antibodies in the CSF/brain). Accordingly, the finding of DeMattos is not teaching that peripheral presence of an A $\beta$  antibody (an APP antibody; i.e. an amyloid binding agent) is the basis for the effects observed *in vivo*.

10. Moreover, it has to be acknowledged that the velocity of A $\beta$  release into the plasma is, in principle, also based on the enhanced presence of high affinity binders in the CNS following application. It is well known to the expert in the field that antibodies to CNS proteins reach the brain very fast after peripheral application (one hour or less) and can thus exert their function *in situ* very early after administration. See the attached publications of Freund (J. Exp. Med. 51 (1930), 889-902) and Masliah et al. (Neuron 46 (2005), 857-868).

11. To interpret the data contained in DeMattos, one would also have to include the extra-neuronal sources of A $\beta$  which are present in the periphery of AD patients (as well as in the transgenic animals in the DeMattos study).

12. The blood and its cellular components are an important source for amyloid peptides. Platelets are well-known to constitute one of the major sources of A $\beta$  in the blood. The antibody presence in the blood can thus also lead to a dramatic release from A $\beta$  from the platelets into the bloodstream accounting at least, in part, for the increase of A $\beta$  reported by DeMattos. Accordingly, the direct contact of high antibody levels with platelets is how the data presented by DeMattos can be reasonably interpreted.

13. In addition it is known that the highly abundant plasma protein albumin is capable of binding the amyloid peptide and is consequently usually associated with A $\beta$  peptides in the circulation. In fact, almost 90% of amyloid peptides in plasma are bound to albumin (see attached article of Biere et al., JBC 271 (1996), 32916-32922).

14. The USPTO on page 5, 2<sup>nd</sup> paragraph of the paper dated May 19, 2010 stated that "the ability to bring A $\beta$  across the membrane is unique to this antibody and it can pull A $\beta$  out of the brain." However, this conclusion is not correct, and even if it was correct this still does

not suggest to one in this field to do what is defined by the method of claim 1 with a reasonable expectation of success.

15. First, DeMattos clearly reports that the effect according to Fig. 1a is also present for other antibodies including m3D6 and m10D5 (see page 8851, left column, 3rd paragraph, last sentence). Moreover, a small amount of the antibody in the brain is sufficient to reduce the very lowly concentrated toxic A $\beta$  species at synapses responsible for disturbing memory formation as described by the article of Dodart et al. (the same group that published DeMattos). As monoclonal antibodies are known to cross the BBB, albeit in low amounts of about 1 to 5%, even this amount is sufficient to clear amyloid deposits during chronic treatment. Moreover, cognitive benefits are detectable before amyloid reduction is detectable in the brain in the model used. This shows that even for DeMattos, one would understand that the effects reported by DeMattos relate to an effect of the antibody in the brain as being important for the overall results.

16. Even if it is true that Fig. 1 A would imply to a person skilled in the art that apheresis with an anti-A $\beta$  antibody would decrease the concentration in the compartment on the other side of the membrane, in the case of the apheresis according to this application, this other side of the membrane would be the human blood and not the human brain. Moreover, it was not shown by DeMattos that the mere reduction of plasma level of A $\beta$  was then sufficient to reduce A $\beta$  in the brain. This is an oversimplification artificially pulled out from this article by the USPTO with the knowledge of having read this application because I certainly do not come to that conclusion when reading DeMattos. In fact, in 2001, DeMattos with the knowledge of the subsequent work of this group, see again Dodart et al, one in this field would know that in addition to the known effects that administration of an antibody in the blood system has in the clearance of the compound to which this antibody binds (i.e. to A $\beta$ ) this antibody also exerts direct effects in the brain. This was regarded as essential for the

effect of the administration of this antibody to the mouse model. Although being administered to the periphery, the antibody evidently crosses the BBB and has effects there which were decisive for the overall effect reported (see Dodart et al. 2002).

17. Kojima shows that an increased plasma level of p2-MG can be reduced in hemodialysis patients (see table 1 of Kojima). However, this reduced level in HD patients is still significantly higher than normal levels (see also table 1 of Kojima). IgE or SAP levels in plasma are shown to be decreased by applying the method. However, Kojima does not contain any information that amyloid depositions in certain compartments or even in the brain decrease with this method. Even if Kojima had shown reduction of several amyloid depositions, this would still only be a reduction within the blood system, i.e., in the periphery.

18. Claim 1 of this application fundamentally differs from Kojima's approach, because apheresis is applied to the blood or plasma flow to reduce amyloid deposition, including APP, in the brain (and not in plasma). Plasma of AD patients does not show an elevated A $\beta$  level. Quite in contrast, DeMattos shows a rapid 1000-fold increase in plasma A $\beta$ . Even though, a corresponding  $\beta$ 2-MG antibody has not been tested in Kojima, it would be expected that such rapid increase in  $\beta$ 2-MG does not occur, because the biology of the proteins in Kojima differs so fundamentally from A $\beta$  used in the context of this application.

19. It is clear to me that DeMattos differs fundamentally from the manner and goal that claim 1 of this application sets out to treat AD and the addition of Kojima simply is not combinable with DeMattos. Even if one did combine those references, there would not have been a reasonable expectation of success due in large part to the significant and fundamental differences between  $\beta$ 2-MG as taught in Kojima compared to the A $\beta$  in the claims of this application.

20. The undersigned declares further that all statements made herein are of his own knowledge, are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

\_\_\_\_\_  
Eliezer Masliah, M.D.

\_\_\_\_\_  
October 24, 2010  
Date

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.  
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME <b>Eliezer Masliah, M.D.</b>		POSITION TITLE <b>Professor of Neurosciences and Pathology</b>	
eRA COMMONS USER NAME <b>Masliah</b>			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
National Institute of Pediatrics, Mexico City, Mexico		1982-1983	Soc. Serv. in Pediatric Pathology
National Autonomous University of Mexico City, Mexico	M.D.	1983	Medicine

**A. Personal Statement**

The main objectives of the research at my laboratory of Experimental Neuropathology at UCSD are to better understand the molecular mechanisms of synaptic pathology in neurodegenerative disorders and to develop new experimental therapies for Alzheimer's Disease and Parkinson's Disease. Alterations in the rate of *aggregation, synthesis or clearance* can result in the formation of toxic oligomers, neurodegeneration and further propagation of the disease. For this reason efforts in my laboratory have been directed at better understanding the mechanisms of oligomers formation and clearance of Abeta and synuclein. Therefore, strategies directed at promoting clearance of oligomers might be of therapeutic value for Alzheimer's Disease and Parkinson's Disease.

**Positions and Honors****Positions and Employment**

1983-1986 Resident, Anatomic Pathology, National Institute of Nutrition, Mexico City, Mexico  
 1988-1990 Postdoctoral Fellow, Pathology of Neurodegenerative Diseases, University of California San Diego, La Jolla, California  
 1990-1991 Assistant Research Neuroscientist, University of California San Diego, La Jolla, California  
 1991-1993 Assistant Professor, Departments of Neurosciences and Pathology, University of California San Diego, La Jolla, California  
 1993-1996 Associate Professor, Departments of Neurosciences and Pathology, University of California San Diego, La Jolla, California  
 1997-present Professor, Departments of Neurosciences and Pathology, University of California San Diego, La Jolla, California  
 1995-present Director of Autopsy Service, Department of Pathology, University of California San Diego, La Jolla, California

**Other Experience and Professional Memberships**

1990- Member, American Association of Neuropathologists  
 1995- Member, Society for Neurosciences  
 1996-00 Member, NIH Neuroscience of Aging Review Committee  
 2007- Member, NIH Cellular and Molecular Biology of Neurodegeneration Study Section  
 2010- Member, Medical and Scientific Advisory Council of the Alzheimer's Association

**Honors**

1988 Weil Award (Honorable Mention), American Association of Neuropathologists  
 1989 Alzheimer Association/George F. Berlinger Faculty Scholar Award  
 1990 Weil Award (Honorable Mention), American Association of Neuropathologists



2006 MERIT Award NIH-NIA

2010 The Dorothy Russell Memorial Medal, British Neuropathology Society

**B. Selected peer-reviewed publications** (Selected from 454 peer-reviewed publications).**Most relevant to the current application**

1. Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Lee M, Chilcote T, Games D, Schenck D. Effects of  $\alpha$ -synuclein immunizations in a mouse model of Parkinson's disease. *Neuron* 46:857-68, 2005. PMID: 15953415
2. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441:513-7, 2006. PMID: 16724068
3. Tsigelny IF, Crews L, Desplats P, Shaked GM, Sharikov Y, Mizuno H, Spencer B, Rockenstein E, Trejo M, Platoshyn O, Yuan JX, Masliah E. Mechanisms of hybrid oligomer formation on the pathogenesis of combined Alzheimer's and Parkinson's Disease. *PLoS ONE*, 9:e3135, 2008. PMID: 18769546
4. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E, Lee SJ. Inclusion formation and neuronal cell death through neuron-to neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A*. 106(31):13010-5, 2009. PMID: 19651612
5. Spencer B, Potkar R, Trejo M, Rockenstein E, Patrick C, Gindi R, Adame A, Wyss-Coray T, Masliah E. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body disease. *J Neurosci*. 28;29(43):13578-88, 2009. PMID: 19864570

**Additional recent publications of importance to the field (in chronological order)**

1. Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, Masliah E. Neuroprotective effects of regulators of the glycogen synthase kinase-3 $\beta$  signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. *J Neurosci* 27:1981-91, 2007. PMID: 18627032
2. Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, Keller SH, Platoshyn O, Yuan JX, Masliah E. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. *FEBS J* 274:1862-77, 2007. PMID: 17381514
3. Belinson H, Lev D, Masliah E, Michaelson DM. Activation of the amyloid cascade in apolipoprotein E4 transgenic mice induces lysosomal activation and neurodegeneration resulting in marked cognitive deficits. *J Neurosci* 28:4690-701, 2008. PMID: 18448646
4. Crews L, Mizuno H, Desplats P, Rockenstein E, Adame A, Patrick C, Winner B, Winkler J, Masliah E. Alpha-synuclein alters Notch-1 expression and neurogenesis in mouse embryonic stem cells and in the hippocampus of transgenic mice. *J Neurosci* 16:4250-60, 2008. PMID: 18417705
5. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, Wyss-Coray T. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest* 118:2190-99, 2008. PMID: 18497889
6. Maranguri R, Spencer B, Crews L, Adame A, Patrick C, Trejo M, Dallapiccola B, Valente EM, Masliah E. Mutant Pink1 induces mitochondrial dysfunction in a neuronal cell model of Parkinson's disease by disturbing calcium flux. *J Neurochem*. 6:1561-74, 2009. PMID: 19166511
7. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med*. 3:331-7, 2009. PMID: 19198615
8. Rose JB, Crews L, Rockenstein E, Adame A, Mante M, Hersh LB, Gage FH, Spencer B, Potkar R, Marr RA, Masliah E. Neuropeptide Y fragments derived from neuropeptide processing are neuroprotective in a transgenic model of Alzheimer's disease. *J Neurosci*. 4:1115-25, 2009. PMID: 19176820
9. Shankar GM, Leissring MA, Adame A, Sun X, Spooner E, Masliah E, Selkoe DJ, Lemere CA, Walsh DM. Biochemical and immunohistochemical analysis of an Alzheimer's disease mouse model reveals the presence of multiple cerebral A $\beta$  assembly forms throughout life. *Neurobiol Dis*. 36:293-302, 2009. PMID: 19660551

10. Cohen E, Paulsson JF, Blinder P, Burstyn-Cohen T, Du D, Estepa G, Adame A, Pham HM, Holzenberger M, Kelly JW, Masliah E and Dillin A. Reduced IGF-1 Signaling Delays Age-associated Proteotoxicity in Mice. Cell. 139:1157-69, 2009. PMID: 20005808

### C. Research Support

#### Ongoing

2 P50 AG05131 (Galasko, Douglas) 05/01/2004 - 03/31/2014  
NIH  
Alzheimer's Disease Research Center - D: Neuropathology Core / 2: Testing the Role of Transport Disturbance in AD  
The major goal focuses on early diagnosis and tracking of Alzheimer's disease as well as potential mechanisms of neurodegeneration and repair.  
Role: PI-Neuropathology Core

2 P01 DA12065 (Grant, Igor) 05/15/2005 - 04/30/2010  
NIH  
NeuroAids: Effects of Methamphetamine and HCV - 6: Neurobiology  
The major goal of this project is to examine neuropathological alteration and patterns of neurodegeneration associated with methamphetamine use in the context of HIV infection.  
Role: PI - Neurobiology Core

5 R01 MH073510 (McGrath, Michael) 02/14/2005 - 01/31/2010  
NIH/University of California, San Francisco  
HIV Sequence Evolution in AIDS Dementia  
The major goal is to investigate the contribution of macrophage subpopulation to the neurodegenerative process in HAD.  
Role: PI - Subcontract

1 R01 NS050041 (Gonzalez, Gilberto) 03/01/2005 - 02/28/2010  
NIH/Mass General Hosp  
Advanced MR Spectroscopy of SIV Brain Injury  
The major goal of this grant is to assess the neurological and neuron damage as well as quantification of macrophages in the brain.

5 P30 MH62512 (Grant, Igor) 04/01/2006 - 03/31/2011  
NIH  
HIV Neurobehavioral Research Center - C: Neurobiology Core  
The major goal is to examine the development and course of neurocognitive & neuropsychiatric consequence of HIV disease.  
Role: PI - Neurobiology Core

1 R01 MH79881-01 (Everall, Ian) 09/27/2006 - 07/31/2011  
NIH  
Somatostatin & Stress-Related Genes in HIV & Comorbid Major Depressive Disorder  
To determine the biological basis for co-occurrence of major depressive disorder in the setting of HIV infection based on brain gene expression, molecular and cellular pathology.  
Role: Co-Investigator

5 R37 AG18440 (Masliah, Eliezer) 09/01/2001 - 08/31/2011  
NIH  
Alpha-Synuclein Clearance Strategies as a Treatment for Lewy Body Disease  
The major goal of this project is to ascertain the anti-aggregations potential of  $\alpha$ -synuclein as a therapeutic target for the development treatment of Parkinson's disease.

Role: PI

5 P30 NS057096 (Lipton, Stuart)

09/01/06 – 08/31/2011

NIH/Burnham Institute

The major goal of this project is to provide to investigators in the La Jolla Neurosciences community a set of technical and neurobiological resources that allows analysis of the degenerative processes associated with neurological conditions.

Role: PI of Neuropathology Core

5 R01 MH62962 (Masliah, Eliezer)

04/01/2007 – 03/31/2012

NIH

Mechanisms of Neuroprotection in HIV Encephalitis

The major goals are to determine patterns expression in the brains of AIDS patients are associated with neuroprotection vs. neurodegeneration.

Role: PI

5 R37 AG11385 (Mucke, Lennart)

06/15/2002 – 03/31/2012

NIH/Gladstone Inst

Transgenic Models to Study Alzheimer's Disease

The major goal is to develop new transgenic models of Alzheimer's disease to investigate amyloid toxicity.

Role: PI – Subcontract

## CURRICULUM VITAE

**Eliezer Masliah, M.D.**

**Date of Birth:** December 9, 1958, Mexico City, Mexico  
**Residence Status:** U.S. Citizen  
**Marital Status:** Married  
**Address:** 4661 Corte Mar Del Corazon, San Diego 92130

### Education:

1977 - 1982 **National Autonomous University of Mexico** Mexico City, Mexico  
School of Medicine  
Doctor of Medicine

1982 - 1983 **National Institute of Pediatrics** Mexico City, Mexico  
Social Service in Pediatric Pathology

### Residencies:

1983 - 1984 **National Institute of Nutrition** Mexico City, Mexico  
General Pathology

1984 - 1985 **National Institute of Cancerology** Mexico City, Mexico  
Oncologic Pathology

1985 - 1986 **National Institute of Pediatrics** Mexico City, Mexico  
Pediatric Pathology

### Postdoctoral Training:

1988 - 1990 **University of California, San Diego** La Jolla, California  
Department of Neurosciences  
Pathology of Neurodegenerative Diseases

### Academic Appointments:

1990 - 1991 **University of California, San Diego** La Jolla, California  
Department of Neurosciences  
Assistant Research Neuroscientist

1991 - 1993 **University of California, San Diego** La Jolla, California  
Departments of Neurosciences and Pathology  
Assistant Professor in Residence

1993 - 1994 **University of California, San Diego** La Jolla, California  
Departments of Neurosciences and Pathology  
Associate Professor in Residence

1994 - 1997 **University of California, San Diego** La Jolla, California  
Departments of Neurosciences and Pathology

Associate Professor (Tenured Track)

1997 -	<b>University of California, San Diego</b> Departments of Neurosciences and Pathology Professor	La Jolla, California
1995-	<b>University of California, San Diego</b> Medical Center, Department of Pathology Director of Autopsy Service	San Diego, California

**Board Certifications:**

1986	Mexican Board of Anatomic Pathology	
1989	Anatomic Pathology Specialty National Autonomous University of Mexico	Mexico City, Mexico

**License:**

<b>Department of Public Education</b> General Department of Professions, License No. 873217	Mexico City, Mexico
<b>Medical Board of California</b> State of California, License No. A067390	California, USA

**Laboratory Experience:**

1979 - 1986	<b>National Autonomous University of Mexico</b> School of Medicine Laboratory Instructor in Human Histology	Mexico City, Mexico
1987 - 1988	<b>University of California, San Diego</b> Department of Neurosciences Laboratory Assistant in Neuropathology	La Jolla, California

**Teaching Experience:**

1979 - 1986	<b>National Autonomous University of Mexico</b> School of Medicine Laboratory Instructor in Histology, Embryology and Genetics	Mexico City, Mexico
1990 - 1995	<b>University of California, San Diego</b> Department of Neurosciences Instructor in Mammalian Neuroanatomy Laboratory (SOM256)	La Jolla, California
1992 - 1998	<b>University of California, San Diego</b> Department of Pathology Instructor in Neuropathology (portion of SOM208)	La Jolla, California
1997 -	<b>University of California, San Diego</b> Department of Pathology Instructor (portion of Path221 & PTH220)	La Jolla, California

**Scientific Associations:**

American Association of Neuropathologists  
Society for Neurosciences

**Honors and Awards:**

1989 Honorable Mention for the Weil Award. American Association of Neuropathologists  
1990 Alzheimer Association/George F. Berlinger Faculty Scholar Award  
1993 Honorable Mention for the Weil Award. American Association of Neuropathologists

**Other:**

1992 - present Ad Hoc Reviewer NINDS/NIH  
1996 - present Member, Neuroscience of Aging Review Committee, NIA/NIH  
1996 - 2000 Associate Editor, Neuroscience Letters  
1998 - present Member, Editorial Board, Journal of Neuropathology & Experimental Neurology  
1998 - present Member, Editorial Board, Laboratory Investigation  
1999 - present UCSD, Cadaver use advisory review committee  
2001 - present UCSD, Dean's SOM Space Advisory Committee  
2002 - present UCSD, Academic Senate  
2002- present ADRC/UCI Advisory Board Committee  
2002- present ADRC/UCSF Advisory Board Committee  
2004 - present UCSD, Search Committee, Department of Pathology  
2006 - present UCSD Committee on Research

**Research Support:**

<b><u>Completed</u></b> <b><u>Funding Agency</u></b> <b><u>Costs</u></b>	<b><u>Grant/Award Number</u></b>	<b><u>Project Title</u></b>	<b><u>Award Period</u></b>	<b><u>Yearly Direct</u></b>
NIH/NIA	1 RO1 AG8201 (PI-Minority investigator research supplement initiative)	Structure and function in Alzheimer Disease	1/1/90-3/31/93	268,584
NIH/NIA	1 RO1 AG10689 (PI)	Subcellular basis of synaptic pathology Alzheimer disease	9/29/91-6/30/94	242,003

Alzheimer Assoc.	FS-90-008 (PI)	Structural and quantitative synaptic changes in relation to amyloid Alzheimer disease	1/1/92-12/31/93	40,835
Calif. State, Dept. Of Health	92-15936 93-18642 94-20619 (PI)	Role of amyloid precursor protein in the synaptic organization of the Neocortex in transgenic mouse models	12/1/92-11/30/95	197,916
Am. Health Assist. Foundation	---- 81,911 (CO-PI)	Cloning of a novel protein of AD amyloid		4/1/93-3/31/94
NIH/NINDS	RO1 NS33056 (PI-subcontract Gladstone Institute)	In vivo CNS effects of HIV coat and regulatory proteins	4/1/94-2/28/98	70,694
NIH/NIA	5 P50 AGO5131 (PI-Project 4)	Alzheimer's Disease Research Center (Synaptic Alterations in APP Transgenic Mice)	4/1/94-3/31/96	134,958
NIH/NIA	5 P50 AGO5131 (CO-PI Neuropath Core)	Alzheimer's Disease Research Center	4/1/94-3/31/99	824,452
NIH/NIMH	5 P50 MH45294 129,445 (PI Neuropath Core)	HIV Neurobehavioral Research	12/1/95-11/30/00	6/1/94-11/30/95 571,567
Athena Neurosciences/ Lilly Pharmaceutical	---- 23,179 (PI)	Ultrastructural alterations in PDAPP tg mouse		7/1/94-12/31/96
NIH/NIA	2 RO1 AG10689-S1 (PI-supplement)	Subcellular basis of synaptic pathology in Alzheimer's disease	9/30/94-6/30/96	109,083
NIH/NINDS (Gladstone Inst)	RO1 NS34602 (PI-subcontract)	Transgenic models to study AIDS/dementia complex	7/1/95-5/31/99	93,686
EBEWE-Arzneimittel	--- 164,485 (PI)	Neuroprotective role of Cerebrolysin in transgenic Models of Alzheimer's disease		12/01/95-5/14/99
Alzheimer's Assoc. 136,365(PI)	IIRG-95-007 150,000	ApoE role in synaptogenesis and neurodegeneration in Alzheimer Disease		7/1/96-6/30/98

Ruth K. Broad Fdn ---- (Duke University) (PI)		Synaptotrophic effects of ApoE	7/1/96-6/30/98	94,090
NIH (Massachusetts General Hospital) (PI-subcontract)	1 RO1 NS34626 198,802	Molecular neuroimaging of AIDS dementia: animal models		1/1/97-6/30/99
Athena Neurosciences----	31,735	Ultrastructural alterations in PD APP mouse		1/1/97-12/31/98
NIH/NIA	2 RO1 AG10689 (PI)	Subcellular basis of synaptic Pathology in Alzheimer disease	1/1/97-11/30/01	549,391
NIH/NIA	5 RO1 AG05131 (PI-supplement Project 4)	Alzheimer's Disease Research Center (Synaptic alterations in APP transgenic mice)	4/1/97-3/31/99	248,488
NIH/NIA	5 P50 AG05131 (PI-pilot project)	Molecular rearrangements of alpha-1 antichymotrypsin and their effects on biological functions of serpin: relevance to Alzheimer's disease pathogenesis	4/1/97-3/31/98	18,000
Stein Inst. (UCSD) --- (PI)		Novel role of RAP in neuro-transmitter modulation: implications for aging and AD	7/1/97-6/40/98	10,000
NIH/NIMH (Scripps)	RO1 MH57061-02 (PI-subcontract)	Borna disease virus and neuropsychiatric disorders	12/1/97-11/30/00	60,000
NIH/NIMH	R24-MH59745 (PI-Neuropathology Unit)	California NeuroAIDS Tissue Network	9/30/98-9/29/03	653,095
NIH/NIDA	P01-DA 12065 505,005 (PI-Project 4)	Mechanisms of Neurodegeneration in METH dependent HIV+ Positive		4/1/99-3/31/04
NIH (Massachusetts General Hospital)	R01 RR13213 (PI-subcontract)	Advanced MR spectroscopy of SIV brain injury	12/1/97-11/30/02	60,000
NIH/NIA	5 P50 AGO5131	Alzheimer's Disease Research	4/1/99-3/31/04	370,000



	(PI-Project 4)	Center (NACP Transgenic Mice)		
NIH/NIA	5 P50 AGO5131 (CO-PI Neuropath Core)	Alzheimer's Disease Research Center	4/1/99-3/31/04	625,000
NIH/NINDS (Gladstone Inst)	AG11385 (PI-subcontract)	Transgenic models of Alzheimer's disease	4/1/98-3/31/99	156,783
NIH/NIA (Supplement)	3 R01 AG11385 (PI-subcontract Gladstone Institute)	Transgenic models to study Alzheimer's disease	10/1/96-6/30/97	50,002
NIH/NINDS (Gladstone Institute)	MH58164-02 (PI-subcontract)	Therapeutic targets in tg mice neurotoxicity in tg mice	7/1/97-6/30/01	120,000
MJ Fox Foundation	(PI)	Transgenic models for treatment of Park Dis	6/1/01-5/31/02	50,000
Proteotech	(PI)	Anti-amyloid.... in APP tg models	1/1/01-5/30/01	50,000
NIH	5 R01 AG10689-08 (PI)	Basis of Synaptic Pathology in Alzheimer's disease	1/1/97-11/30/02	111,809
NIH	5 R01 NS34626-07 (PI)	Molecular Neuroimaging of AIDS Dementia: Animal Models	5/1/99-4/30/03	19,161

**Current**

<b><u>Funding Agency</u></b>	<b><u>Grant/Award Number</u></b>	<b><u>Project Title</u></b>	<b><u>Award Period</u></b>	<b><u>Yearly Direct Costst</u></b>
NIH	5 P30 MH62512 (PI -Neurobiology Core)	HIV Neurobehavioral Research Center - CC: Neurobiology Core	04/01/06 – 03/31/11	\$177,825
NIH	2 R24 MH59745 (PI - Project 8)	NeuroAIDS Tissue Network	09/30/98 - 05/31/03	\$131,482
NIH	5 R37 AG18440 (PI)	Alpha-synuclein clearance strategies as a Treatment for Lewy Body Disease	09/01/01 - 08/31/11	\$250,000
NIH	5 R01 MH62962 (PI)	Mechanisms of Neuroprotection in HIV Encephalitis	02/20/02 – 03/31/12	\$250,000
NIH (Gladstone Inst)	5 P01 AG022074 (PI- Subcontract)	Proteopathies of the Aging Central Nervous System	06/15/03 - 05/31/08	\$144,4810
NIH (Gladstone Inst)	5 R37 AG11385 (PI - Subcontract)	Transgenic Models to Study Alzheimer's Disease	06/15/02 - 03/31/12	\$90,472

NIH (Salk Institute)	5 P01 AG10435 (PI- Subcontract)	Lentivirus for Modeling and Treatment of AD in TG Mice	09/30/02 - 08/31/07	\$157,345
NIH	5 P01 NS044233 (Co-PI - Project 3)	Pathogenesis and Diagnosis of Multiple System Atrophy- Project 3	09/30/03 - 06/30/08	\$144,481
Elan Pharmaceuticals Inc	PI	Development of an Anti-Parkinson's Vaccine in Transgenic Mice	04/01/04 - 03/31/08	\$80,000
NIH	2 P50 AG05131 (PI - Project 4)	Alzheimer's Disease Research Center - D: Neuropathology Core/2: Testing the Role of Transport Disturbance in AD	05/01/04 - 03/31/09	\$206,817
NIH	2 P01 DA12065 (PI-Neuropath)	NeuroAIDS: Effects of Methamphetamine - Neuropathology/ Neuropathology Supplement	07/01/04 - 06/30/09	\$108,951
US-Israel Binational Science Foundation	Stanford University/ Larry Hillblom Foundation	Isoform Specific Effects of Apolipoprotein E on the Aggregation, Deposition and Toxicity of Beta-amyloid in vivo	10/01/04 - 09/30/08	\$10,000
NIH (University of California, San Francisco)	PY-2598 (PI - Subcontract)	Synaptic Structure and Function in Down Syndrome	01/01/05 - 12/31/06	\$22,000
Elan Pharmaceuticals Inc	5 R01 MH073510 (PI- Subcontract)	HIV Sequence Evolution in AIDS Dementia	02/14/05 - 01/31/10	\$250,000
UC Discovery Grant / Kinexis Inc.	PI	Role of Mononuclear Leukocytic Infiltration in the Early Neuro-Inflammatory Response in Patients with Alzheimer's Disease and Lewy body dementia	07/01/05 - 06/30/06	\$70,000
Michael J. Fox / Proteotech	bio05-10557 (PI - Subcontract)	Alzheimer's vaccine that avoids inflammatory side effects.	09/01/06 - 08/31/08	\$75,000
NIH (Mass General Hospital)	(PI-Subcontract)	New small molecule inhibiting agents of alpha-synuclein and Lewy body formation as disease-modifying treatments for Parkinson's disease	01/01/06-12/31/08	\$96,000
	(PI-Subcontract)	Advanced MR Spectroscopy of SIV Brain Injury	03/01/05 -02/28/10	\$217,469

# **List of Students:**

## **Pathology Residents:**

Jennifer Strother	1996-97
Warren Snider	1996-
Barney Welsh	1996-
TJ Bisla	1996-
Swen Holland	1996-97
Harper Summers	1996-
Cathy Palmer	1997
Siria Poucell	1997;
Vivian Tan	1997-
Slav Uglik	1997-98
Peilin Zhang	1997-98
Min Huang	1997-98
Phil Cacheris	1998
Serguei Bannykh	1998-02
Wenxi Wang	1998-99
Hongchen Jia	1998-99
Mark Lu	1998-02
Dawn Jacobson	1998-02
Galina Bannykh	1999-02
Lisa Albers	1999-02
Michan Afsari	1999-02
Keith Overstreet	2000-02
Helena Whang	2000-02
Shawn Emery	2000-01
Emma Du	1999-02
Kelli Hanson	1999-02
Anna Moyahan	2000-01
Chris Wixom	2000-01
Chris Holt	2001-02
Joseph Hughes	2001-02
Linda Roley	2001-02
Ayce Unal	2001-02
Truc Pham	2002-03; 05
Mark Butler	2002-03;
Omid Bakhtar	2002-03;
Nicole Nilson	2002-03; 05
Carter Wahl-	2002-03;
Chris Wixom	2002-03
Susan Duncan	2002-03
Joan Bernard	2003-04
Joe Hughes	2003-04
Arman Kasian	2003-04
Grace Lin	2003-04
Mike Peterson	2003-04
Curtis Strong	2003-04
Suchi Pandey	2003-04
Kyle Noskoviak	2003-04
Mark Peterson	2003-04
Owan Chan	2003-04
Sima Fargahi	2004-05
Ryan Olson	2004-05
Richard Scuder	2005-05

## **Graduate students:**

Karen Huang	01/94 -
David Kang	04/94 -
Silvia Tara	06/95 -
Emily Van Uden	04/96 -
Christine McGiffert	09/96 -
Lynn Weber	09/96 -
Sarah Moskowitz	01/97 -
Helen Le-Nicolescu	01/97 -
Dan Kalafus	01/97 -
Aaron Bowman	01/97 -
Keith Magert	01/97 -
Jason Greenwald	01/97 -
Leigh Hsu	06/96 -
Isaac Veinbergs	06/96 -
Kelly Chang	01/99 -
David Merriam	04/99 -
David Moore	03/01 -
Jon Violin	10/99 -
Gudren Wakonning	02/99 -
Jason Freshwater	04/00 -
Dan Oral	02/00 -
Ruth Carper	08/00 -
Paula Soto	02/01 -
Brian Kasper	07/01 -
Zhen Li	05/01 -
Laura Rassor	11/01 -
Jorge Munera	08/05 -
Leslie Crews	09/05 -
Erin Conn	06/06 -
Roberta Marongiu	04/06 -

## **Undergraduate students:**

Robert Johnson	04/91 -
Archana Jaiswal	04/92 -
Isaac Veinbergs	04/92 -
Mitchell Voight	04/92 -
Mario Cole	10/92 -
Karina Herrera	07/93 -
Mark Smith	02/94 -
Shelly Banerjee	06/94 -
Julio Diaz	06/94 -
Mike Mante	01/95 -
Lon Tran	04/95 -
Laura Fox	08/95 -
Abraham Hanono	10/95 -
Arie Yafeh	03/96 -
Mario Manese	04/97 -
Jennifer Mattli	06/98 -
Michael Kim	01/99 -
Gabriel Larrea	12/00 -
Matthew Izu	07/02 -
Timothy Wilson	4/05-6/05

## **Medical Students:**

Lida Ghaderi	09/91 -
Matt Kodosi	05/91 -
John Leake	10/92 -
Ivan Burgos	05/93 -
Elana Lehman	04/94 -
Ji Yoo	09/95 -
Mark Tiffany	03/96 -
Shawn Emery	09/99 -
Patricia Hackett	09/99 -
Jason Himmel	07/01 -
Brian Webster	06/03 -
Jennifer Kum	01/04 -
Jason Tovar	6/02 -
Kathy Rameriz	02/03 -
Geoffrey Biard	05/03 -
Mike Cho	06/03 -

## **MD/PhD Students:**

Peter Nakaji	10/92 -
Matt Kodosi	04/95 -
Albert Lai	01/95 -

## **Post-doctoral Fellows:**

Nianfeng Ge	06/93 -
Virginia Sanders	
Shi Li	01/96 -
William Samuel	04/95 -
Veronika Thoms	06/96 -
Makoto Hashimoto	05/96 -
Gilbert Ho	09/98 -
Takato Takenouchi	12/98 -
Avako Takeda	08/97 -
Dianne Langford	06/00 -
Ingrid Lang	06/98 -
David Song	07/01 -
Dalen Agnew	11/01 -
Jeannelyn Estrella	06/05 -
Pazit Bar-On	09/02 -
Aline Grigorian	09/02 -
Hideya Mizuno	11/04 -
Gideon Shakked	07/06 -
Shigeto Sato	10/06 -
Andrew Koob	01/07 -
Paula Desplats	05/07 -

## **Visiting Scholars:**

Koichi Wakabayashi	1994
Ian Everall	1996 - 97,
Federico Licastro	1996 - 97,
Jack de la Torre	1999 - 00
Gudrun Wakonigg	1998 - 99
Ingrid Lang	1999
Seigo Tanaka	1999 - 00
Koichi Kawahara	2003 -
Phil Hyu Lee	2007 -

## PUBLICATIONS:

### Professional Thesis:

1. For the MD Degree: "Identification of GFAP in tumors of the nervous system in children". National Institute of Pediatrics and National Autonomous University of Mexico, Mexico City, Mexico. 1982
2. For the Pathology Specialty: "Morphometric Studies in Pathobiology". National Institute of Nutrition/ National Autonomous University of Mexico, Mexico City, Mexico. 1987

### Research Articles:

#### 1981-1987

1. Reyes M, **Masliah E**, Besthoff L. [Neuroblastoma. Recent Concepts.] Rev Fac Med Mex 24:19-34, 1981 (Spa)
2. **Masliah E**, Perez-Tamayo R. [A Note on the Histopathology of Invasive Amebiasis of the Large Bowel.] Patologia (Mex) 22:233-45, 1984. (Eng abstract). (Spa)
3. **Masliah E**, Perez-Tamayo R. [Submassive Hepatic Necrosis. Quantification of the hepatic damage and correlation with clinical data.] Patologia (Mex) 23:39-46, 1985. (Eng abstract) (Spa)
4. **Masliah E**. A unified hypothesis for the origin of soft tissue sarcomas of unknown origin. Med Hypoth 20:393-401, 1986.
5. **Masliah E**. [A new thermodynamic theory to explain life under normal and pathological conditions.] Patologia (Mex) 24: 79, 1986 (Letter) (Spa).
6. Mora-Tiscareno A, **Masliah E**, Gonzalez J, Ramirez J, Meneses A. [Cervico-uterine carcinoma: study of 56 autopsy cases]. Rev INC (Mex) 33:470-8, 1987 (Eng abst) (Spa).

#### 1989-1990

7. Cole G, **Masliah E**, Huynh TV, DeTeresa R, Terry RD, Okuda C, Saitoh T. An antiserum against amyloid  $\beta$ -protein precursor detects a unique peptide in Alzheimer brain. Neurosci Lett 100:340-6, 1989. [PMID: 2503790]
8. **Masliah E**, Terry RD, Buzsaki G. Thalamic nuclei in Alzheimer disease: evidence against the cholinergic hypothesis of plaque formation. Brain Res 493:240-6, 1989. [PMID: 2765898]
9. Hansen L, **Masliah E**, Terry RD, Mirra SS. A neuropathological subset of Alzheimer's disease with concomitant Lewy body disease and spongiform change. Acta Neuropathol 78:194-201, 1989. [PMID: 2546359]
10. **Masliah E**, Terry RD, DeTeresa R, Hansen LA. Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease. Neurosci Lett 103:234-9, 1989. [PMID: 2505201]
11. **Masliah E**, Terry RD, Alford M, DeTeresa R. Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. J Histochem Cytochem 38:837-44, 1990. [PMID: 2110586]
12. Iimoto DS, **Masliah E**, DeTeresa R, Terry RD, Saitoh T. Aberrant casein kinase II in Alzheimer disease. Brain Res 507:273-80, 1990. [PMID: 2110586]

13. Hansen L, Salmon D, Galasko D, **Masliah** E, DeTeresa R, Thal L, Katzman R, Pay M, Hofstetter R, Klauber M, Rice V, Butters N, Alford M. The Lewy body variant of Alzheimer's disease: a clinical and pathological entity. Neurology 40:1-8, 1990. [PMID: 2153271]
14. **Masliah** E, Cole G, Shimohama S, Hansen L, DeTeresa R, Terry RD, Saitoh T. Differential involvement of protein kinase C isozymes in Alzheimer's disease. J Neurosci 10:2113-24, 1990. [PMID: 2376771]
15. **Masliah** E, Galasko D, Wiley CA, Hansen LA. Lobar atrophy with dense core (brainstem type) Lewy bodies in a patient with dementia. Acta Neuropathol 80:453-8, 1990. [PMID: 2173332]
16. Ueda K, **Masliah** E, Saitoh T, Bakalis SL, Scoble H, Kosik KS. Alz-50 recognizes a phosphorylated epitope of tau protein. J Neurosci 10:3295-304, 1990. [PMID: 1698946]
17. **Masliah** E, Iimoto DS, Saitoh T, Hansen LA, Terry RD. Increased immunoreactivity of brain spectrin in Alzheimer disease: a marker of synapse loss? Brain Res 531:36-44, 1990. [PMID: 2289136]
18. **Masliah** E, Terry RD, Mallory M, Alford M, and Hansen LA. Diffuse plaques do not accentuate synapse loss in Alzheimer's disease. Am J Pathol 137:1293-7, 1990. [PMID: 2124413]

#### 1991-1993

19. **Masliah** E, Terry RD, Alford M, DeTeresa R, Hansen LA. Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer disease. Am J Pathol 138:235-46, 1991. [PMID: 1899001]
20. **Masliah** E, Hansen LA, Quijada S, DeTeresa R, Alford M, Kauss J, Terry R. Late onset dementia with argyrophilic grains and subcortical tangles or atypical progressive supranuclear palsy? Ann Neurol 29:389-96, 1991. [PMID: 1929210]
21. Shapiro I P, **Masliah** E, Saitoh T. Altered protein tyrosine phosphorylation in Alzheimer's disease. J Neurochem 56:1152-62, 1991. [PMID: 1705956]
22. **Masliah** E, Hansen L, Albright T, Mallory M, Terry RD. Immunoelectron microscopic study of synaptic pathology in Alzheimer disease. Acta Neuropathol 81:428-33, 1991. [PMID: 1903014]
23. **Masliah** E, Yoshida K, Shimohama S, Gage F H, Saitoh T. Differential expression of protein kinase C isozymes in rat primary glial cultures. Brain Res 549:106-11, 1991. [PMID: 1893244]
24. Cole GM, **Masliah** E, Shelton ER, Chan H, Terry RD, Saitoh T. Accumulation of amyloid precursor fragment in Alzheimer plaque. Neurobiol Aging 12: 85-91, 1991. [PMID: 1711161]
25. Wiley CA, **Masliah** E, Morey M, Lemere C, DeTeresa R, Grafe M, Hansen L, Terry RD. Neocortical damage during HIV infection. Ann Neurol 29:651-7, 1991. [PMID: 1909852]
26. Saitoh T, **Masliah** E, Jin L-W, Cole GM, Wieloch T, Shapiro P. Protein kinases and phosphorylation in neurological disorders and cell death. Lab Invest 64:596-616, 1991. [PMID: 2030574]
27. **Masliah** E, Mallory M, Hansen L, Alford M, Albright T, DeTeresa R, Terry RD, Baudier J, Saitoh T. Patterns of aberrant sprouting in Alzheimer disease. Neuron 6:729-39, 1991. [PMID: 1827266]
28. Hansen LA, Natelson BH, Lemere C, Nieman W, DeTeresa R, Regan TJ, **Masliah** E, Terry RD. Alcohol-induced brain changes in dogs. Arch Neurol 48:939-42, 1991. [PMID: 1953418]

29. **Masliah E**, Fagan AM, Terry RD, DeTeresa R, Mallory M, Gage FH. Reactive synaptogenesis assessed by synaptophysin immunoreactivity is associated with GAP-43 in the dentate gyrus of the adult rat. Exp Neurol 113:131-42, 1991. [PMID: 1831150]
30. Armstrong TP, Hansen LA, Salmon DP, **Masliah E**, Pay M, Kunin JM, Katzman R. Rapidly progressive dementia in a patient with the Lewy body variant of Alzheimer's disease. Neurology 41:1178-80, 1991. [PMID: 1714056]
31. **Masliah E**, Cole GM, Hansen LA, Mallory M, Albright T, Terry RD, Saitoh T. Protein kinase C alteration is an early biochemical marker in Alzheimer's disease. J Neurosci 11:2759-67, 1991. [PMID: 1880547]
32. Terry RD, **Masliah E**, Salmon D, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572-80, 1991. [PMID: 1789684]
33. **Masliah E**, Hansen L, Mallory M, Albright T, Terry RD. Abnormal brain spectrin immunoreactivity in sprouting neurons in Alzheimer disease. Neurosci Lett 129:1-5, 1991. [PMID: 1922957]
34. Buzsaki G, **Masliah E**, Chen LS, Horvath Z, Terry RD, Gage FH. Hippocampal grafts into the intact brain induce epileptic patterns. Brain Res 554:30-7, 1991. [PMID: 1933310]
35. Hansen LA, **Masliah E**, Quijada-Fawcett S, Rexin D. Entorhinal neurofibrillary tangles in Alzheimer disease with Lewy bodies. Neurosci Lett 129:269-72, 1991. [PMID: 1745407]
36. **Masliah E**, Mallory M, Hansen L, Alford M, Albright T, Terry RD, Shapiro P, Sundsmo M, Saitoh T. Immunoreactivity of CD45, a protein tyrosine phosphatase, in Alzheimer's disease. Acta Neuropathol 83:12-20, 1991. [PMID: 1388850]
37. **Masliah E**, Ge N, Morey M, DeTeresa R, Terry RD, Wiley CA. Cortical dendritic pathology in human immunodeficiency virus encephalitis. Lab Invest 66:285-91, 1992. [PMID: 1538584]
38. Baum L, **Masliah E**, Iimoto DS, Hansen L, Halliday WC, Saitoh T. Casein kinase II is associated with neurofibrillary tangles but is not an intrinsic component of paired helical filaments. Brain Res 573:126-32, 1992. [PMID: 1576530]
39. **Masliah E**, Iimoto DS, Mallory M, Albright T, Hansen L, Saitoh T. Casein kinase alteration precedes tau accumulation in tangle formation. Am J Pathol 140:263-8, 1992. [PMID: 1739121]
40. **Masliah E**, Mallory M, Hansen L, Alford M, DeTeresa R, Terry RD, Baudier J, Saitoh T. Localization of amyloid precursor protein in GAP43-immunoreactive aberrant sprouting neurites in Alzheimer's disease. Brain Res 574:312-6, 1992. [PMID: 1386275]
41. **Masliah E**, Ellisman M, Carragher B, Mallory M, Young S, Hansen L, DeTeresa R, Terry RD. Three-dimensional analysis of the relationship between synaptic pathology and neuropil threads in Alzheimer disease. J Neuropathol Exp Neurol 51:404-14, 1992. [PMID: 1619440]
42. **Masliah E**, Achim CL, Ge N, DeTeresa R, Terry RD, Wiley CA. Spectrum of HIV associated neocortical damage. Ann Neurol 32:321-9, 1992. [PMID: 1416802]
43. Hessler D, Young SJ, Carragher BO, Martone M, Lamont S, Whittaker M, Milligan RA, **Masliah E**, Hinshaw JE, Ellisman M. Programs for visualization in three-dimensional microscopy. NeuroImage 1:55-67, 1992. [PMID: 9343557]
44. **Masliah E**, Mallory M, Ge N, Saitoh T. Amyloid precursor protein is localized in growing neurites of neonatal rat brain. Brain Res 593:323-8, 1992. [PMID: 1450940]

45. Connor DJ, Thal LJ, Mandel RJ, Langlais PL, **Masliah E**. Independent effects of age and nucleus basalis magnocellularis lesion: maze learning, cortical neurochemistry, and morphometry. Beh Neurosci 106:776-88, 1992. [PMID: 1280146]
46. **Masliah E**, Ge N, Achim C, Hansen LA, Wiley CA. Selective neuronal vulnerability in HIV encephalitis. J Neuropathol Exp Neurol 51:585-93, 1992. [PMID: 1484289]
47. **Masliah E**, Mallory M, Ge N, Saitoh T. Amyloid precursor protein is localized in growing neurites of neonatal rat brain. Brain Res 593:323-8, 1992. [PMID: 1450940]
48. Buzaki G, Hsu M, Horvath Z, Horsburgh K, Sundsmo M, **Masliah E**, Saitoh T. Kindling-induced changes of protein kinase C levels in hippocampus and neocortex. Epilepsy Res Suppl 9:279-83, 1992. [PMID: 1337442]
49. Saitoh T, **Masliah E**, Baum L, Sundsmo M, Flanagan L, Vikramkumar R, Kay M. Degradation of proteins in the membrane-cytoskeletal complex in Alzheimer's disease. Might amyloidogenic APP processing be just the tip of the iceberg? Ann NY Acad Sci 674:180-92, 1992. [PMID: 1288363]
50. **Masliah E**, Mallory M, Hansen L, DeTeresa R, Terry RD. Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43:192-7, 1993. [PMID: 8423884]
51. **Masliah E**, Mallory M, Hansen L, Alford M, DeTeresa R, Terry RD. An antibody against phosphorylated neurofilaments identifies a subset of damaged associated axons in Alzheimer's disease. Am J Pathol 142:871-82, 1993. [PMID: 8456946]
52. **Masliah E**, Terry RD. The role of synaptic proteins in the pathogenesis of disorders of the central nervous system. Brain Pathol 3:77-85, 1993. [PMID: 8269086]
53. Lev-Ram V, Valsamis M, **Masliah E**, Levine S, Godfrey HP. A novel non-ataxic guinea pig strain with cerebrocortical and cerebellar abnormalities. Brain Res 606:325-31, 1993. [PMID: 8490725]
54. **Masliah E**, Mallory M, DeTeresa R, Alford M, Hansen L. Differing patterns of aberrant neuronal sprouting in Alzheimer disease with and without Lewy bodies. Brain Res 617:258-66, 1993. [PMID: 8402154]
55. **Masliah E**, Miller A, Terry RD. The synaptic organization of the neocortex in Alzheimer's disease. Med Hypoth 41:334-40, 1993. [PMID: 8289698]
56. Reyes E, Gamboa A, **Masliah E**. Atypical Lewy body disease with neuritic abnormalities. Clin Neuropathol 12:330-4, 1993. [PMID: 8287626]
57. Langlais PJ, Thal L, Hansen L, Galasko D, Alford M, **Masliah E**. Neurotransmitters in basal ganglia and cortex of Alzheimer's disease with and without Lewy bodies. Neurology 43:1927-34, 1993. [PMID: 8105420]
58. **Masliah E**, Mallory M, Alford M, Saitoh T. Immunoreactivity of the nuclear antigen p105 is associated with tangle and plaque formation in Alzheimer's disease. Lab Invest 69:562-9, 1993. [PMID: 8246448]
59. **Masliah E**, Mallory M, Ge N, Godson C, Saitoh T. Phorbol ester-induced neuritic alterations in the rat neocortex. Structural and immunocytochemical studies. Mol Chem Neuropath 20:125-45, 1993. [PMID: 8297418]
60. **Masliah E**, Mallory M, Deerinck T, DeTeresa R, Lamont S, Miller A, Terry RD, Carragher B, Ellisman M. Re-evaluation of the organization of neuritic plaques in Alzheimer's disease. J Neuropathol Exp Neurol 52:619-32, 1993. [PMID: 8229081]

61. Ueda K, Fukushima H, **Masliah E**, Xia Y, Iwai A, Otero D, Kondo J, Ihara Y, Saitoh T. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci USA 90:11282-6, 1993. [PMID: 8248242]
62. Hansen LA, **Masliah E**, Galasko D, Terry RD. Plaque only Alzheimer disease is usually the Lewy body variant, and vice versa. J Neuropathol Exp Neurol 52:648-54, 1993. [PMID: 8229084]
63. Campbell I, Abraham CR, **Masliah E**, Kemper Ph, Inglis JD, Oldstone MBA, Mucke L. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin-6. Proc Natl Acad Sci USA 90:10061-5, 1993. [PMID: 7694279]
64. Nagra RM, **Masliah E**, Wiley C. Synaptic and dendritic pathology in murine retroviral encephalitis. Exp Neurol 124:283-8, 1993. [PMID: 8287927]
65. Saitoh T, Horsburg K, **Masliah E**. Hyperactivation of signal transduction systems in Alzheimer's disease. Ann NY Acad Sci 695:34-41, 1993. [PMID: 8239309]
66. **Masliah E**, Terry R. The role of synaptic pathology in the mechanisms of dementia in Alzheimer's disease. Clin Neurosci 1:192-8, 1993.
67. Toggas SM, **Masliah E**, Rockenstein EM, Rall GF, Abraham CR, Mucke L. Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. Nature 367:188-93, 1994. [PMID: 8114918]
68. Alford MF, **Masliah E**, Hansen L, Terry RD. A simple dot-immunobinding assay for quantification of synaptophysin-like immunoreactivity in human brain. J Histochem Cytochem 42:283-7, 1994. [PMID: 8288869]
69. **Masliah E**, Honer WG, Mallory M, Voigt M, Kushner P, Terry R. Topographical distribution of synaptic-associated proteins in the neuritic plaques of Alzheimer disease hippocampus. Acta Neuropathol 87:135-42, 1994. [PMID: 8171963]
70. Roch J-M, **Masliah E**, Roch-Levecq AC, Sundsmo M, Otero D, Veinbergs I, Saitoh T. Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid  $\beta$ /A4. Proc Natl Acad Sci USA 91:7650-4, 1994. [PMID: 8052602]
71. **Masliah E**, Mallory M, Hansen L, DeTeresa R, Alford M, Terry RD. Synaptic and neuritic alterations during the progression of Alzheimer's disease. Neurosci Lett 174:67-72, 1994. [PMID: 7970158]
72. Samuel W, Terry RD, DeTeresa R, Butters N, **Masliah E**. Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. Arch Neurol 51:772-8, 1994. [PMID: 8042925]
73. Bowes MP, **Masliah E**, Chen B-L, Otero D, Zivin J, Saitoh T. Reduction of neurological damage by a trophic peptide segment of the amyloid  $\beta$ /A4 protein (APP). Exp Neurol 129:1-8, 1994. [PMID: 7925833]
74. Mucke L, **Masliah E**, Johnson WB, Ruppe M, Alford M, Rockenstein EM, Forss-Petter S, Pietropaolo M, Mallory M, Abraham C. Synaptotrophic effects of human amyloid beta protein precursors in the cortex of transgenic mice. Brain Res 666:151-67, 1994. [PMID: 7882025]
75. Samuel W, **Masliah E**, Terry R. Hippocampal connectivity and Alzheimer's dementia: effects of pathology in a two-component model. Neurology 44:2081-8, 1994. [PMID: 7969963]
76. Galasko D, Hansen LA, Katzman R, Wiederholt W, **Masliah E**, Terry R, Hill LR, Lessin P, Thal LJ. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 51:888-95, 1994. [PMID: 8080388]



77. Heindel WC, Jernigan TL, Archibald SL, Achim CL, **Masliah E**, Wiley C. The relationship of quantitative brain magnetic resonance imaging measures to neuropathological indexes of HIV infection. Arch Neurol 51:1129-35, 1994. [PMID: 7980109]
78. Reyes E, Mohar A, Mallory M, Miller A, **Masliah E**. Hippocampal involvement associated with human immunodeficiency virus in Mexico. Arch Pathol Lab Med 118:1130-4, 1994. [PMID: 7979899]
79. **Masliah E**, Ge N, Achim CL, Wiley CA. Cytokine receptor alterations during HIV infection in the human central nervous system. Brain Res 663:1-6, 1994. [PMID: 7850458]
80. Nagra RM, **Masliah E**, Burrola PG, Wiley CA. Neocortical pathology in chronic murine retroviral encephalitis. J Neuropathol Exp Neurol 53:572-81, 1994. [PMID: 7964898]
81. Jin L-W, Ninomiya H, Roch J-M, Schubert D, **Masliah E**, Otero DAC, Saitoh T. Peptides containing RERMS sequence of amyloid beta/A4 protein precursor bind cell surface and promote neurite extension. J Neurosci 14:5461-70, 1994. [PMID: 8083748]
82. Wakabayashi K, Honer WG, **Masliah E**. Synapse alterations in the hippocampal-entorhinal formation in Alzheimer's disease with and without Lewy body disease. Brain Res 667:24-32, 1994. [PMID: 7895080]
83. Wiley CA, **Masliah E**, Achim CL. Measurement of CNS HIV burden and its association with neurologic damage. Adv Neuroimm 4:319-25, 1994. [PMID: 7874400]
84. Saitoh T, Xia Y, **Masliah E**, Terry R, Galasko D, Shults C, Hill R, Thal L, Hansen L, Katzman R. Association of the CYP2D6B mutant allele with Lewy body variant of Alzheimer's disease. Ann Neurol 37:110-2, 1995. [PMID: 7818242]
85. Iwai A, **Masliah E**, Yoshimoto M, de Silva R, Saitoh T. The precursor protein of non-A $\beta$  component of Alzheimer's disease amyloid (NAC) is a presynaptic protein of the central nervous system. Neuron 14:467-75, 1995. [PMID: 7857654]
86. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, **Masliah E**, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J. Development of neuropathology similar to Alzheimer's disease in transgenic mice overexpressing the V717F  $\beta$ -amyloid precursor protein. Nature 373:523-7, 1995. [PMID: 7845465]
87. **Masliah E**. The natural evolution of the neurodegenerative alterations in Alzheimer's disease. Neurobiol Aging 16:280-2, 1995.
88. Mucke L, Abraham CR, Rupprecht MD, Rockenstein EM, Toggas SM, Alford M, **Masliah E**. Protection against gp120-induced brain damage by neuronal expression of human APP. J Exp Med 181:1551-6, 1995. [PMID: 7699335]
89. Wakabayashi K, Hansen LA, **Masliah E**. Cortical Lewy body-containing neurons are pyramidal cells: laser confocal imaging of double-immunolabeled sections with ubiquitin and SMI32. Acta Neuropathol 89:404-8, 1995. [PMID: 7618438]
90. **Masliah E**, Ge N, Achim CL, Wiley CA. Differential vulnerability of calbindin-immunoreactive neurons in HIV encephalitis. J Neuropathol Exp Neurol 54:350-7, 1995. [PMID: 7745434]
91. Smith MC, Mallory M, Hansen LA, **Masliah E**. Fragmentation of the neuronal cytoskeleton in the Lewy body variant of Alzheimer's disease. NeuroReport 6:673-6, 1995. [PMID: 7605925]

92. Saitoh T, **Masliah E**. What causes neurofibrillary tangle formation in Alzheimer's disease? Neurobiol Aging 16:418-20, 1995.
93. **Masliah E**. Mechanisms of synaptic dysfunction in Alzheimer's disease. Histol Histopathol 10:509-19, 1995. [PMID: 7599445]
94. Wyss-Coray T, Feng L, **Masliah E**, Lee HS, Ruppe MD, Toggas SM, Rockenstein EM, Mucke L. Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor  $\beta$ 1. Am J Pathol 147:53-67, 1995. [PMID: 7604885]
95. **Masliah E**, Mallory M, Alford M, Hansen L, Saitoh T. PDGF is associated with neuronal and glial alterations of Alzheimer's disease. Neurobiol Aging 16: 549-56, 1995. [PMID: 8544904]
96. Mucke L, **Masliah E**, Campbell IL. Transgenic models to assess the neuropathogenic potential of HIV-1 proteins and cytokines. Curr Topics Microbiol Immunol 202:187-205, 1995. [PMID: 7587363]
97. Iwai A, **Masliah E**, Yoshimoto M, Saitoh T. Non-A $\beta$  component of Alzheimer's disease amyloid (NAC) is amyloidogenic. Biochemistry 34:10139-45, 1995. [PMID: 7640267]
98. Chen KS, **Masliah E**, Mallory M, Gage F. Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human NGF infusion. Neuroscience 68:19-27, 1995. [PMID: 7477924]
99. Burgos I, Cuello AC, Liberini P, Pioro E, **Masliah E**. NGF-mediated synaptic sprouting in the cerebral cortex of lesioned primate brain. Brain Res 692:154-60, 1995. [PMID: 8548299]
100. Thinakaran G, Kitt CA, Roskams AJ, Slunt HH, **Masliah E**, von Koch C, Ginsberg S, Ronnett GV, Reed RR, Price DL, Sisodia SS. Distribution of an APP homologue, APLP2, in the mouse olfactory system: a potential role for APLP2 in axogenesis. J Neurosci 15:6314-26, 1995. [PMID: 7472397]
101. Chen Y, Xia Y, Alford M, DeTeresa R, Hansen L, Klauber MR, Katzman R, Thal L, **Masliah E**, Saitoh T. The CYP2D6B allele is associated with a milder synaptic pathology in Alzheimer's disease. Ann Neurol 38:653-8, 1995. [PMID: 7574463]
102. Okazaki T, **Masliah E**, Wang H, Cao M, Johnson SA, Saitoh T, Mori N. Altered expression of the developmentally regulated neuronal growth-associated protein SCG10 in Alzheimer's disease. Neurobiol Aging 16:883-94, 1995. [PMID: 8622778]
103. **Masliah E**, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD. Neurodegeneration in the CNS of apoE-deficient mice. Exp Neurol 136:107-22, 1995. [PMID: 7498401]
104. Rockenstein EM, McConlogue L, Tan H, Power M, **Masliah E**, Mucke L. Levels and alternative splicing of amyloid  $\beta$  protein precursor (APP) transcripts in brains of APP transgenic mice and humans with Alzheimer's disease. J Biol Chem 270:28257-67, 1995. [PMID: 7499323]
105. **Masliah E**, Ge N, Achim CL, DeTeresa R, Wiley CA. Patterns of neurodegeneration in HIV encephalitis. NeuroAIDS 1:161-73, 1996. [PMID: 16873160]
106. Samuel W, Hansen LA, Galasko D, **Masliah E**. Neocortical Lewy body density correlates with dementia in the Lewy body variant of Alzheimer's disease. J Neuropathol Exp Neurol 55:44-52, 1996. [PMID: 8558171]
107. Mucke L, Abraham CR, **Masliah E**. Neurotrophic and neuroprotective effects of hAPP in transgenic mice. Ann NY Acad Sci 777:82-8, 1996. [PMID: 8624131]

108. Toggas SM, **Masliah E**, Mucke L. Prevention of HIV-1 gp120-induced neuronal damage in the central nervous system of transgenic mice by the NMDA receptor antagonist memantine. Brain Res 706:303-7, 1996. [PMID: 8822372]
109. Wyss-Coray T, **Masliah E**, Toggas SM, Rockenstein EM, Booker MJ, Lee HS, Mucke L. Dysregulation of signal transduction pathways as a potential mechanism of nervous system alterations in HIV-1 gp120 transgenic mice and humans with HIV-1 encephalitis. J Clin Invest 97:789-98, 1996. [PMID: 8609236]
110. **Masliah E**, Iwai A, Mallory M, Ueda K, Saitoh T. Altered presynaptic protein NACP is associated with plaque formation and neurodegeneration in Alzheimer's disease. Am J Pathol 148:201-10, 1996. [PMID: 8546207]
111. Roses AD, Einstein G, Gilbert J, Goedert M, Han S-H, Huang D, **Masliah E**, Pericak-Vance MA, Saunders AM, Schmechel DE, Strittmatter WJ, Wisgraber KH. Morphological, biochemical, and genetic support for a metabolic mechanism of the variable rate of disease expression associated with inheritance of different apolipoprotein E alleles. Ann NY Acad Sci 777:146-57, 1996. [PMID: 8624078]
112. Iwai A, **Masliah E**, Sundsmo M, DeTeresa R, Mallory M, Saitoh T. The synaptic protein NACP is abnormally expressed during the progression of Alzheimer's disease. Brain Res 720:230-4, 1996. [PMID: 8782917]
113. Jin LW, **Masliah E**, Iimoto D, DeTeresa R, Sundsmo M, Mori N, Sobel A, Saitoh T. Neurofibrillary tangle-associated alterations of stathmin in Alzheimer's disease. Neurobiol Aging 17:331-41, 1996. [PMID: 8725893]
114. Achim C, **Masliah E**, Ge N, Heyes M, Wiley C. Macrophage activation factors in the brains of AIDS patients. NeuroAIDS 1:1-16, 1996. [PMID: 16873161]
115. Garcia-Munoz M, Patino P, **Masliah M**, Young SJ, Groves PM. Glutamate-dependent long-term presynaptic changes in corticostriatal excitability. Neuroscience 73:109-19, 1996. [PMID: 8783235]
116. De la Torre JC, Mallory M, Brot M, Gold L, Koob G, Oldstone MBA, **Masliah E**. Viral persistence in neurons alters synaptic plasticity and cognitive functions without destruction of brain cells. Virology 220:508-15, 1996. [PMID: 8661403]
117. **Masliah E**. *In vivo* modeling of HIV-1 mediated neurodegeneration. Am J Pathol 149:745-50, 1996. [PMID: 8780378]
118. **Masliah E**, Alford M, DeTeresa R, Mallory M, Hansen LA. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann Neurol 40:759-66, 1996. [PMID: 8957017]
119. Xia Y, Rohan de Silva HA, Rosi BL, Yamaoka LH, Rimmier J, Pericak-Vance MA, Roses A, Chen X, **Masliah E**, DeTeresa R, Iwai A, Sundsmo M, Hofstetter CR, Gregory E, Hansen L, Katzman R, Thal L, Saitoh T. Genetic studies in Alzheimer's disease with an NACP/ $\alpha$ -Synuclein polymorphism. Ann Neurol 40:207-15, 1996. [PMID: 8773602]
120. **Masliah E**, Ge N, Mucke L. Pathogenesis of HIV-1 associated neurodegeneration. Crit Rev Neurobiol 10:57-67, 1996. [PMID: 8853954]
121. Salmon DP, Galasko D, Hansen LA, **Masliah E**, Butters N, Thal LJ, Katzman R. Neuropsychological deficits associated with diffuse Lewy body disease. Brain Cogn 31:148-65, 1996. [PMID: 8811990]
122. Godson C, **Masliah E**, Balboa MA, Ellisman MH, Insel PA. Isoform-specific redistribution of protein kinase C in living cells. Biochem Biophys Acta 1313:63-71, 1996. [PMID: 8781551]

123. **Masliah E**, Sisk A, Mallory M, Mucke L, Schenk D, Games D. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein and Alzheimer's disease. J Neurosci 16:5795-811, 1996. [PMID: 8795633]
124. **Masliah E**, Mallory M, Veinburg I, Miller A, Samuel W. Alterations in apoE expression during aging and neurodegeneration. Prog Neurobiol 50:493-503, 1996. [PMID: 9015824]
125. de la Torre JC, Gonzalez-Dunia D, Cubitt B, Mallory M, Mueller-Lantzsch N, Grasser FA, Hansen LA, **Masliah E**. Detection of Borna disease virus antigen and RNA in human autopsy brain samples from neuropsychiatric patients. Virology 223:272-82, 1996. [PMID: 8806563]
126. Baum L, Hansen L, **Masliah E**, Saitoh T. Glycogen synthase kinase 3 alterations in Alzheimer disease is related to neurofibrillary tangle formation. Mol Chem Neuropathol 29:253-61, 1996. [PMID: 8971700]

# 1997-1999

127. Corey-Bloom J, Sabbagh MN, Bondi MW, Hansen LA, Alford MF, **Masliah E**, Thal LJ. Hippocampal sclerosis contributes to dementia in the elderly. Neurology 48:154-60, 1997. [PMID: 9008511]
128. Saitoh T, Kang D, Mallory M, DeTeresa R, **Masliah E**. Glial cells in Alzheimer's disease: Preferential effects of APOE risk on scattered microglia. Gerontology 43:109-18, 1997. [PMID: 8996833]
129. Heyser CJ, **Masliah E**, Samimi A, Campbel IL, Gold L. Progressive cognitive decline paralleled by inflammatory neurodegeneration in interleukin-6 transgenic mice expressing IL6 in the brain. Proc Natl Acad Sci USA 94:1500-05, 1997. [PMID: 9037082]
130. Conrad C, Andreadis A, Trojanowski JQ, Dickson DW, Kang D, Chen X, Wiederholt W, Hansen L, **Masliah E**, Thal L, Katzman R, Xia Y, Saitoh T. Genetic evidence for the etiological involvement of tau in progressive supranuclear palsy. Ann Neurol 41:277-81, 1997. [PMID: 9029080]
131. Wakabayashi K, Hansen L, Vincent I, Mallory M, **Masliah E**. Neurofibrillary tangles in the dentate granule cells in Alzheimer's disease, Lewy body disease and progressive supranuclear palsy. Acta Neuropathol 93:7-12, 1997. [PMID: 9006651]
132. **Masliah E**, Westland CE, Rockenstein EM, Abraham CR, Mallory M, Veinbergs I, Sheldon E, Mucke L. Amyloid precursor protein protects neurons of transgenic mice against acute and chronic injuries *in vivo*. Neuroscience 78:135-46, 1997. [PMID: 9135095]
133. **Masliah E**, Samuel W, Veinbergs I, Mallory M, Mante M, Saitoh T. Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. Brain Res 751:307-14, 1997. [PMID: 9099820]
134. Fox L, Mallory M, Achim C, **Masliah E**. Neurodegeneration of somatostatin-immunoreactive neurons in HIV encephalitis. J Neuropathol Exp Neurol 56:360-8, 1997. [PMID: 9100666]
135. Kang DE, Saitoh T, Chen X, Xia Y, **Masliah E**, Hansen LA, Thomas RG, Thal LJ, Katzman R. Genetic association of the low density lipoprotein receptor related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. Neurology 49:56-61, 1997. [PMID: 9222170]
136. Li S, Mallory M, Alford M, Tanaka S, **Masliah E**. Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. J Neuropathol Exp Neurol 56:901-11, 1997. [PMID: 9258260]

137. Hashimoto M, Yoshimoto M, Sisk A, Hsu LJ, Sundsmo M, Kittel A, Saitoh T, Miller A, **Masliah E**. NACP, a synaptic protein involved in Alzheimer's disease, is differentially regulated during megakaryocyte differentiation. Biochem Biophys Res Comm 237:611-6, 1997. [PMID: 9299413]
138. Samuel W, **Masliah E**, Brush E, Garcia-Munoz M, Patino P, Young S, Groves P. Lesions in the dentate Hilum regions of the rat hippocampus produce cognitive deficits that correlate with site-specific glial activation. Neurobiol Learning Mem 68:103-16, 1997. [PMID: 9322254]
139. **Masliah E**. Role of amyloid precursor protein in the mechanisms of neurodegeneration in Alzheimer's disease. Lab Invest 77:197-209, 1997. [PMID: 9314943]
140. Wyss-Coray T, **Masliah E**, Mallory M, McConlogue L, Johnson-Wood K, Lin C, Mucke L. Amyloidogenic role of cytokine TGF- $\beta$ 1 in transgenic mice and in Alzheimer's disease. Nature 389:602-6, 1997. [PMID: 9335500]
141. **Masliah E**, Heaton R, Marcotte TD, Ellis R, Wiley C, Mallory M, Achim CL, McCutchan A, Nelson JA, Atkinson H, Grant I, the HNRC group. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. Ann Neurol 42:963-72, 1997. [PMID: 9403489]
142. Hashimoto M, Inoue S, Muramatsu M, **Masliah E**. Estrogens stimulate tamoxifen-induced neuronal cell apoptosis *in vitro*: A possible nongenomic action. Biochem Biophys Res Comm 240:464-70, 1997. [PMID: 9388502]
143. Thorns V, Mallory M, Hansen L, **Masliah E**. Alterations in glutamate receptor 2/3 subunits and amyloid precursor protein expression during the course of Alzheimer's disease and Lewy body variant. Acta Neuropathol 94:539-48, 1997. [PMID: 9444355]
144. Campbell IL, Stalder AK, Chiang C-S, Bellinger R, Heyser CJ, Steffensen S, **Masliah E**, Powell H, Gold LH, Henriksen SJ, Siggins G. Transgenic models to assess the pathogenic actions of cytokines in the central nervous system. Mol Psych 2:125-9, 1997. [PMID: 9106234]
145. Komori N, Kittel A, Kang D, Shackelford D, **Masliah E**, Zivin JA, Saitoh T. Reversible ischemia increases levels of Alzheimer amyloid precursor protein without increasing levels of mRNA in the rabbit spinal cord. Brain Res Mol Brain Res 49:103-12, 1997. [PMID: 9387869]
146. Everall IP, DeTeresa R, Terry R, **Masliah E**. Comparison of two Quantitative methods for the evaluation of neuronal number in the frontal cortex in Alzheimer disease. J Neuropath Exp Neurol 56: 1202-6, 1997. [PMID: 9370230]
147. Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, **Masliah E**. Abnormal accumulation of NACP/a-synuclein in neurodegenerative disorders. Am J Pathol 152:367-72, 1998. [PMID: 9466562]
148. Licastro F, Mallory M, Hansen LA, **Masliah E**. Increased levels of a-1-antichymotrypsin in brains of patients with Alzheimer's disease correlate with activated astrocytes and are affected by APOE4 genotype. J Neuroimmunol 88:105-10, 1998. [PMID: 9688331]
149. Conrad C, Amano N, Andreadis A, Xia Y, Namekata K, Oyama F, Ikeda K, Wakabayashi K, Takahashi H, Thal LJ, Katzman R, Shackelford DA, Matsushita M, **Masliah E**, Sawa A. Differences in a dinucleotide repeat polymorphism in the tau gene between Caucasian and Japanese populations: implication for progressive supranuclear palsy. Neurosci Lett 250:135-7, 1998. [PMID: 9697937]
150. **Masliah E**. Mechanisms of synaptic pathology in Alzheimer's disease. J Neural Transm [Suppl] 53:147-58, 1998. [PMID: 9700653]

151. Tanaka S, Chen X, Xia Y, Kang D, Matoh N, Sundsmo M, Thomas RG, Katzman R, Thal LJ, Trojanowski JQ, Saitoh T, Ueda K, **Masliah E**. Association of CYP2D microsatellite polymorphism with Lewy body variant of Alzheimer's disease. Neurology 50:1556-62, 1998. [PMID: 9633694]
152. Yi ES, Salgado M, Williams S, Kim S-J, **Masliah E**, Yin S, Ulich TR. Keratinocyte growth factor decreases pulmonary edema, transforming growth factor-beta and platelet-derived growth factor-BB expression, and alveolar type II cell loss in bleomycin-induced lung injury. Inflammation 22:315-25, 1998. [PMID: 9604718]
153. **Masliah E**, Raber J, Alford M, Mallory M, Mattson MP, Yang D, Wong D, Mucke L. Amyloid protein precursor stimulates excitatory amino acid transport. Implications for roles in neuroprotection and pathogenesis. J Biol Chem 273:12548-54, 1998. [PMID: 9575214]
154. **Masliah E**. The role of synaptic proteins in neurodegenerative disorders. NeuroSci News 1:14-20, 1998.
155. Thoms V, Hansen L, **Masliah E**. nNOS expressing neurons in the entorhinal cortex and hippocampus are affected in-patients with Alzheimer's disease. Exp Neurol 150:14-20, 1998. [PMID: 9514829]
156. Wiley CA, Sontornniyomkij V, Radhakrishnan L, **Masliah E**, Mellors J, Hermann SA, Dailey P, Achim CL. Distribution of brain HIV load in AIDS. Brain Pathol 8:277-84, 1998. [PMID: 9546286]
157. Hsu LJ, Mallory M, Xia Y, Veinbergs I, Hashimoto M, Yoshimoto M, Thal LJ, Saitoh T, **Masliah E**. Expression pattern of synucleins (non-A $\beta$  component of Alzheimer's disease amyloid precursor protein/ $\alpha$ -synuclein) during murine brain development. J Neurochem 71:338-44, 1998. [PMID: 9648883]
158. Hashimoto M, Inoue S, Ogawa S, Conrad C, Muramatsu M, Shackelford D, **Masliah E**. Rapid fragmentation of vimentin in human skin fibroblasts exposed to tamoxifen: A possible involvement of caspase-3. Biochem Biophys Res Commun 247:401-6, 1998. [PMID: 9642140]
159. Ginsberg SD, Galvin JE, Chin T-S, Lee VM-Y, **Masliah E**, Trojanowski JQ. RNA sequestration to pathological lesions of neurodegenerative diseases. Acta Neuropathol 96:487-94, 1998. [PMID: 9829812]
160. Buttini M, Westland CE, **Masliah E**, Yafeh AM, Wyss-Coray T, Mucke L. Novel role of human CD4 molecule identified in neurodegeneration. Nature Med 4:441-6, 1998. [PMID: 9546790]
161. Akwa Y, Hassett DE, Eloranta M-L, Sandberg K, **Masliah E**, Powell H, Bloom FE, Whitton JL, Campbell IL. Transgenic expression of IFN- $\gamma$  in the central nervous system of mice protects against lethal neurotropic viral infection but induces inflammation and neurodegeneration. J Immunol 161:5016-26, 1998. [PMID: 9794439]
162. **Masliah E**, Mallory M, Alford M, Tanaka S, Hansen LA. Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer's disease. J Neuropathol Exp Neurol 57:1041-52, 1998. [PMID: 9825941]
163. Sabbagh MN, Reid RT, Corey-Bloom J, Rao TS, Hansen LA, Alford MF, **Masliah E**, Adem A, Lloyd GK, Thal LJ. Correlation of nicotinic binding with neurochemical markers in Alzheimer's disease. J Neural Transm 105:709-17, 1998. [PMID: 9826113]
164. Takeda A, Hashimoto M, Mallory M, Sundsmo M, Hansen L, Sisk A, **Masliah E**. Abnormal distribution of the non-A $\beta$  component of Alzheimer's disease amyloid precursor/a-synuclein in Lewy body disease as revealed by proteinase K and formic acid pretreatment. Lab Invest 78:1169-77, 1998. [PMID: 9759660]
165. Licastro F, Davis LJ, Pedrini S, Galasko D, **Masliah E**. Prostaglandin E2 induced polymerization of human a-1-antichymotrypsin and suppressed its protease inhibitory activity: implications for Alzheimer's disease. Biochem Biophys Res Commun 249:182-6, 1998. [PMID: 9705853]

166. Stalder AK, Carson MJ, Pagenstecher A, Asensio VC, Kincaid C, Benedict M, Powell HC, **Masliah E**, Campbell IL. Late-onset chronic inflammatory encephalopathy in immune-competent and severe combined immune-deficient (SCID) mice with astrocyte-targeted expression of tumor necrosis factor. Am J Pathol 153:767-83, 1998. [PMID: 9736027]
167. Hashimoto M, Hsu LJ, Sisk A, Xia Y, Takeda A, Sundsmo M, **Masliah E**. Human recombinant NACP/ $\alpha$ -synuclein is aggregated and fibrillated in vitro: Relevance for Lewy body disease. Brain Res 799:301-6, 1998. [PMID: 9675319]
168. Veinbergs I, Jung MW, Young SJ, Van Uden E, Groves PM, **Masliah E**. Altered long term potentiation in the hippocampus of apolipoprotein E-deficient mice. Neurosci Lett 249:71-4, 1999. [PMID: 9682819]
169. Chen KS, **Masliah E**, Grajeda H, Guido T, Huang J, Khan K, Motter R, Soriano F, Games D. Neurodegenerative Alzheimer-like pathology in PDAPP 717V-->F transgenic mice. Prog Brain Res 117:327-34, 1998. [PMID: 9932418]
170. **Masliah E**, Amorsolo F, Veinbergs I, Mallory M, Samuel W. Cerebrolysin ameliorates performance deficits and neuronal damage in apolipoprotein E-deficient mice. Pharmacol Biochem Beh 62:239-45, 1999. [PMID: 9972690]
171. Van Uden E, Veinbergs I, Mallory M, Orlando R, **Masliah E**. A novel role for receptor-associated protein in somatostatin modulation: Implications for Alzheimer's disease. Neuroscience 88:687-700, 1999. [PMID: 10363810]
172. Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sundsmo M, **Masliah E**. Oxidative stress induces amyloid-like aggregate formation of NACP/ $\alpha$ -synuclein in vitro. NeuroReport 10:717-21, 1999. [PMID: 10208537]
173. Emery SC, Karpinski NC, Hansen L, **Masliah E**. Abnormalities in central nervous system development in osteogenesis imperfecta type II. Pediatr Dev Pathol 2:124-30, 1999. [PMID: 9949218]
174. Reynolds WF, Rhees J, Maciejewski D, Paladino T, Sieburg H, Maki RA, **Masliah E**. Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. Exp Neurol 155:31-41, 1999. [PMID: 9918702]
175. Strother J, Fedullo P, Yi ES, **Masliah E**. Complex vascular lesions at autopsy in a patient with phentermine-fenfluramine use and rapidly progressing pulmonary hypertension. Arch Pathol Lab Med 123:539-40, 1999. [PMID: 10383810]
176. Thorns V, **Masliah E**. Evidence for neuroprotective effects of acidic fibroblast growth factor in Alzheimer's disease. J Neuropathol Exp Neurol 58:296-306, 1999. [PMID: 10197821]
177. Everall IP, Heaton RK, Marcotte T, Ellis RJ, McCutchan JA, Atkinson JH, Grant I, Mallory M, **Masliah E**, and the HNRC Group. Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. Brain Pathol 9:209-17, 1999. [PMID: 10219738]
178. Veinbergs I, **Masliah E**. Synaptic alterations in apolipoprotein E knockout mice. Neuroscience 91:401-3, 1999. [PMID: 10336088]
179. Hsia AY, **Masliah E**, McConlogue L, Yu G-Q, Tatsuno G, Hu K, Kholodenko D, Malenka RC, Nicoll RA, Mucke L. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. Proc Natl Acad Sci USA 96:3228-33, 1999. [PMID: 10077666]
180. Powell HC, Garrett RS, Brett FM, Chiang CS, Chen E, **Masliah E**, Campbell IL. Response of glia, mast cells and the blood brain barrier, in transgenic mice expressing interleukin-3 in astrocytes, and experimental model for CNS demyelination. Brain Pathol 9:219-35, 1999. [PMID: 10219739]

181. Baum L, Chen L, **Masliah E**, Chan YS, Ng HK, Pang CP. Lipoprotein lipase mutations and Alzheimer's disease. Am J Med Genet 88:136-9, 1999. [PMID: 10206232]
  182. Mallory M, Honer W, Hsu L, Johnson R, Rockenstein E, **Masliah E**. In vitro synaptotrophic effects of Cerebrolysin in NT2N cells. Acta Neuropathol 97:437-46, 1999. [PMID: 10334480]
  183. Veinbergs I, Mallory M, Mante M, Rockenstein E, Gilbert JR, **Masliah E**. Differential neurotrophic effects of apolipoprotein E in aged transgenic mice. Neurosci Lett 265:218-22, 1999. [PMID: 10327170]
  184. Veinbergs I, Mante M, Jung MW, Van Uden E, **Masliah E**. Synaptotagmin and synaptic transmission alterations in apolipoprotein E-deficient mice. Prog Neuro-Psychopharmacol Biol Psych 23:519-31, 1999. [PMID: 10378234]
  185. Hashimoto M, **Masliah E**.  $\alpha$ -synuclein in Lewy body disease and Alzheimer's disease. Brain Pathol 9:707-20, 1999. [PMID: 10517509]
  186. Licastro F, Campbell IL, Kincaid C, Veinbergs I, Van Uden E, Rockenstein E, Mallory M, Gilbert JR, **Masliah E**. A role for apoE in regulating the levels of alpha-1-antichymotrypsin in the aging mouse brain and in Alzheimer's disease. Am J Pathol 155:869-75, 1999. [PMID: 10487844]
  187. Van Uden E, Carlson G, Mallory M, Rockenstein E, Orlando R, **Masliah E**. Aberrant Presenilin-1 expression downregulates LDL receptor-related protein (LRP): is LRP central to Alzheimer's disease pathogenesis? Mol Cell Neurosci 14:129-40, 1999. [PMID: 10479411]
  188. Wiley CA, Achim CL, Christopherson C, Kidane Y, Kwok S, **Masliah E**, Mellors J, Radhakrishnan L, Wang G, Soontornniyomkij V. HIV mediates a productive infection of the brain. AIDS 13:2055-9, 1999. [PMID: 10546857]
  189. Hashimoto M, Takeda A, Hsu LJ, Takenouchi T, **Masliah E**. Role of cytochrome c as a stimulator of  $\alpha$ -synuclein aggregation in Lewy body disease. J Biol Chem 274:28849-52, 1999. [PMID: 10506125]
  190. Zhang P, **Masliah E**. Adrenal vein thromboses in an infant of diabetic mother. Pediatr Dev Pathol 2:570-3, 1999. [PMID: 10508881]
  191. Sabbagh MN, Corey-Bloom J, Tiraboschi P, Thomas R, **Masliah E**, Thal LJ. Neurochemical markers do not correlate with cognitive decline in the Lewy body variant of Alzheimer disease. Arch Neurol 56:1458-61, 1999. [PMID: 10593300]
  192. Woo S-I, Hansen LH, Yu X, **Masliah E**. Alternative splicing patterns of CYP2D genes in Lewy body disease. Neurology 53:1570-2, 1999. [PMID: 10534269]
  193. Czygan M, Hallensleben W, Hofer M, Pollak S, Sauder C, Bilzer T, Blumcke I, Riederer P, Bogerts B, Falkai P, Schwarz MJ, **Masliah E**, Staeheli P, Hufert FT, Lieb K. Borna disease virus in human brains with a rare form of hippocampal degeneration, but not in brains of patients with common neuropsychiatric disorders. J Inf Dis 180:1695-9, 1999. [PMID: 10515835]
- 2000**
194. Takeda A, Hashimoto M, Mallory M, Sundsmo M, Hansen L, **Masliah E**. C-terminal  $\alpha$ -synuclein immunoreactivity in structures other than Lewy bodies in neurodegenerative disorders. Acta Neuropathol 99:296-304, 2000. [PMID: 10663973]



195. Licastro F, **Masliah E**, Pedrini S, Thal LJ. Blood levels of  $\alpha$ -1-antichymotrypsin and risk factors for Alzheimer's disease: effects of gender and apolipoprotein E genotype. Dement Geriatr Cogn Disord 11:25-8, 2000. [PMID: 10629358]
196. Sanders VJ, Everall IP, Johnson R, **Masliah E**. Fibroblast growth factor modulates HIV co-receptor CXCR4 expression by neural cells. J Neurosci Res 59:671-9, 2000. [PMID: 10686595]
197. Gonzalez-Dunia D, Watanabe M, Syan S, Hans A, Mallory M, Brahic M, **Masliah E**, de la Torre JC. Synaptic pathology in Borna disease virus persistent infection. J Virol 74:3441-8, 2000. [PMID: 10729116]
198. **Masliah E**, DeTeresa R, Mallory M, Hansen LA. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. AIDS 14:69-74, 2000. [PMID: 10714569]
199. **Masliah E**, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke M. Dopaminergic loss and Inclusion body formation in a-synuclein mice: implications for neurodegenerative disorders. Science 287:1265-9, 2000. [PMID: 10678833]
200. Tiraboschi P, Hansen LA, Alford M, **Masliah E**, Thal LJ, Corey-Bloom J. The decline in synapses and cholinergic activity is asynchronous in Alzheimer's disease. Neurology 55:1278-83, 2000. [PMID: 11087768]
201. Corey-Bloom J, Tiraboschi P, Hansen LA, Alford M, Schoos B, Sabbagh MN, **Masliah E**, Thal LJ. E4 allele dosage does not predict cholinergic activity or synaptic loss in Alzheimer's disease. Neurology 54:403-6, 2000. [PMID: 10668702]
202. Poucell-Hatton S, Huang M, Bannykh S, Benirschke K, **Masliah E**. Fetal obstructive uropathy: patterns of renal pathology. Pediatr Dev Pathol 3:223-31, 2000. [PMID: 10742409]
203. Mucke L, **Masliah E**, Yu G-Q, Mallory M, Tatsuno G, Hu K, Kholodenko D, McConlogue L. High- level neuronal expression of A $\beta$ 1-42 in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. J Neurosci 20:4050-68, 2000. [PMID: 10818140]
204. **Masliah E**, Alford M, Mallory M, Rockenstein E, Moechars D, Van Leuven F. Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. Exp Neurol 163:381-7, 2000. [PMID: 10833311]
205. Plannels-Cases R, Caprini M, Zhang J, Rockenstein EM, Rivera RR, Murre C, **Masliah E**, Montal M. Neuronal death and perinatal lethality in voltage-gated sodium channel  $\alpha$ -11-deficient mice. Biophys J 78:2878-91, 2000. [PMID: 10827969]
206. **Masliah E**, Rockenstein E. Genetically altered transgenic models of Alzheimer's disease. J Neural Transm Suppl 59:175-83, 2000. [PMID: 10961430]
207. Veinbergs I, Mante M, Mallory M, **Masliah E**. Neurotrophic effects of Cerebrolysin in animal models of excitotoxicity. J Neural Transm 59:273-80, 2000. [PMID: 10961439]
208. Hashimoto M, Takenouchi T, Mallory M, **Masliah E**, Takeda A. The role of NAC in amyloidogenesis in Alzheimer's disease. Am J Pathol 156:734-6, 2000. [PMID: 10667911]
209. Lashley DB, **Masliah E**, Kaplan GW, McAleer IM. Megacystis microcolon intestinal hypoperistalsis syndrome: bladder distension and pyelectasis in the fetus without anatomic outflow obstruction. Urology 55:774iv-774vi, 2000. [PMID: 10792103]

210. Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, Wong J, Takenouchi T, Hashimoto M, **Masliah E.**  $\alpha$ -Synuclein promotes mitochondrial deficit and oxidative stress. Am J Pathol 157:401-10, 2000. [PMID: 10934145]
  211. Van Uden E, Kang DE, Koo EH, **Masliah E.** LDL receptor-related protein (LRP) in Alzheimer's disease: Towards a unified theory of pathogenesis. Microsc Res Tech 50:268-72, 2000. [PMID: 10936878]
  212. Van Uden E, Sagara Y, Van Uden J, Orlando R, Mallory M, Rockenstein E, **Masliah E.** A protective role of the low density lipoprotein receptor-related protein against amyloid  $\beta$ -protein toxicity. J Biol Chem 275:30525-30, 2000. [PMID: 10899157]
  213. Veinbergs I, Mallory M, Sagara Y, **Masliah E.** Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E deficient mice. Eur J Neurosci 12:1-8, 2000. [PMID: 11122365]
  214. **Masliah E.** The role of synaptic proteins in Alzheimer's disease. Ann NY Acad Sci 924:68-75, 2000. [PMID: 11193804]
  215. Gonzalez RG, Cheng LL, Westmoreland SV, Sakaie KE, Becerra LR, Lee PL, **Masliah E,** Lackner A. Early brain injury in the SIV-macaque model of AIDS. AIDS 14:2841-9, 2000. [PMID: 11153665]
  216. Wiley CA, Achim CL, Hammond R, Love S, **Masliah E,** Radhakrishnan L, Sanders V, Wang G. Damage and repair of DNA in HIV encephalitis. J Neuropathol Exp Neurol 59:955-65, 2000. [PMID: 11089573]
  217. Yi ES, Kim H, Ahn H, Strother J, Morris T, **Masliah E,** Hansen LA, Park K, Friedman PJ. Distribution of obstructive intimal lesions and their cellular phenotypes in chronic pulmonary hypertension. A morphometric and immunohistochemical study. Am J Respir Crit Care Med 162:1577-86, 2000. [PMID: 11029379]
  218. Welsh JB, Yi ES, Pretorius DH, Scioscia A, Mannino FL, **Masliah E.** Amnion rupture sequence and severe congenital high airway obstruction. J Perinatol 20:387-9, 2000. [PMID: 11002880]
  219. Schiffer HH, Swanson GT, **Masliah E,** Heinemann SF. Unequal expression of allelic kainate receptor GluR7 mRNAs in human brains. J Neurosci 20:9025-33, 2000. [PMID: 11124978]
  220. Mucke L, Yu G-Q, McConlogue L, Rockenstein EM, Abraham CR, **Masliah E.** Astroglial expression of human  $\alpha$ -1-antichymotrypsin enhances Alzheimer-like pathology in amyloid protein precursor transgenic mice. Am J Pathol 157:2003-10, 2000. [PMID: 11106573]
  221. Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos B, **Masliah E,** Thal LJ, Corey-Bloom J. Cholinergic dysfunction in diseases with Lewy bodies. Neurology 54:407-11, 2000. [PMID: 10668703]
  222. Wyss-Coray T, Lin C, Sanan DA, Mucke L, **Masliah E.** Chronic overproduction of transforming growth factor- $\beta$ 1 by astrocytes promotes Alzheimer's disease-like microvascular degeneration in transgenic mice. Am J Pathol 156:139-50, 2000. [PMID: 10623661]
- 2001**
223. Hsu L, Rockenstein E, Mallory M, Hashimoto M, **Masliah E.** Altered expression of glutamate transporters under hypoxic conditions in vitro. J Neurosci Res 64:193-202, 2001. [PMID: 11288147]
  224. Everall IP, Trillo-Pazos G, Bell C, Mallory M, Sanders V, **Masliah E.** Amelioration of neurotoxic effects of HIV envelope protein gp120 by fibroblast growth factor: a strategy for neuroprotection. J Neuropathol Exp Neurol 60:293-301, 2001. [PMID: 11245213]

225. Bannykh SI, Bannykh GI, Mannino FL, Jones KL, Hansen L, Benirschke K, **Masliah E**. Partial caudal duplication in a newborn associated with meningocele and complex heart anomaly. [Teratology](#) 63:94-9, 2001. [PMID: 11241432]
226. Zhukareva V, Vogelsberg-Ragaglia V, Van Deerlin VM, Bruce J, Shuck T, Grossman M, Clark CM, Arnold SE, **Masliah E**, Galasko D, Trojanowski JQ, Lee VM. Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. [Ann Neurol](#) 49:165-75, 2001. [PMID: 11220736]
227. Yeon SW, Jung MW, Ha MJ, Kim SU, Huh K, Savage MJ, **Masliah E**, Mook-Jung I. Blockade of PKC epsilon activation attenuates phorbol ester-induced increase of  $\alpha$ -secretase-derived secreted form of amyloid precursor protein. [Biochem Biophys Res Commun](#) 280:782-7, 2001. [PMID: 11162589]
228. Takenouchi T, Hashimoto M, Hsu LJ, Mackowski B, Rockenstein E, Mallory M, **Masliah E**. Reduced neuritic outgrowth and cell adhesion in neuronal cells transfected with human  $\alpha$ -synuclein. [Mol Cell Neurosci](#) 17:141-50, 2001. [PMID: 11161475]
229. **Masliah E**, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW Jr, Morris JC. Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. [Neurology](#) 56:127-9, 2001. [PMID: 11148253]
230. **Masliah E**, Sisk A, Mallory M, Games D. Neurofibrillary pathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein. [J Neuropathol Exp Neurol](#) 60:357-68, 2001. [PMID: 11305871]
231. **Masliah E**. Recent advances in the understanding of the role of synaptic proteins in Alzheimer's disease and other neurodegenerative disorders. [J Alz Dis](#) 3:121-9, 2001. [PMID: 12214081]
232. Wyss-Coray T, Lin C, Yan F, Yu G-Q, Rohde M, McConlogue L, **Masliah E**, Mucke L. TGF $\beta$ 1 promotes microglial amyloid- $\beta$  clearance and reduces plaque burden in transgenic mice. [Nature Med](#) 7:612-8, 2001. [PMID: 11329064]
233. Langford TD, **Masliah E**. Crosstalk between components of the blood brain barrier and cells of the CNS in microglial activation in AIDS. [Brain Pathol](#) 11:306-12, 2001. [PMID: 11414473]
234. Du EZ, Yung GL, Le DT, **Masliah E**, Yi ES, Friedman PJ. Severe alveolar proteinosis following chemotherapy for acute myeloid leukemia in a lung allograft recipient: A case report. [J Thoracic Imaging](#) 16:307-9, 2001. [PMID: 11685098]
235. Surguchov A, McMahan B, Campbell R, **Masliah E**, Surgucheva I. Synucleins in ocular tissues. [J Neurosci Res](#) 65:68-77, 2001. [PMID: 11433431]
236. Morgello S, Gelman BB, Kozlowski P, **Masliah E**, Cornford M, Vinters HV, Cavert W, Rausch D. The national NeuroAIDS tissue consortium: A new paradigm in brain banking with an emphasis on infectious disease. [Neuropathol Appl Neurobiol](#) 27:326-35, 2001. [PMID: 11532163]
237. **Masliah E**, Ho G, Wyss-Coray T. Functional role of TGF $\beta$  in Alzheimer's disease microvascular injury: Lessons from transgenic mice. [Neurochem Int](#) 39:393-400, 2001. [PMID: 11578774]
238. Veinbergs I, Van Uden E, McGiffert C, Mallory M, DeTeresa R, **Masliah E**. Role of apolipoprotein E receptors in regulating the differential *in vivo* neurotrophic effects of apolipoprotein E. [Exp Neurol](#) 170:15-26, 2001. [PMID: 11421580]
239. **Masliah E**, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, Mucke L.  $\beta$ -amyloid peptides enhance  $\alpha$ -synuclein accumulation and neuronal deficits in a transgenic model linking Alzheimer's disease and Parkinson's disease. [Proc Natl Acad Sci USA](#) 98:12245-50, 2001. [PMID: 11572944]

240. Rockenstein E, Hansen L, Mallory M, Trojanowski JQ, Galasko D, **Masliah E**. Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease. Brain Res 914:48-56, 2001. [PMID: 11578596]
  241. Hashimoto M, Rockenstein E, Mante M, Mallory M, **Masliah E**.  $\beta$ -Synuclein inhibits  $\alpha$ -synuclein aggregation: possible role as an anti-parkinsonian factor. Neuron 32:213-23, 2001. [PMID: 11683992]
  242. Chen Q, Yoshida H, Kimura H, Schubert D, Maher P, Mallory M, **Masliah E**. Presenilin binding protein is associated with neurofibrillary alterations in Alzheimer's disease and stimulates tau phosphorylation. Am J Pathol 159:1597-602, 2001. [PMID: 11696419]
  243. Benirschke K, **Masliah E**. The placenta in multiple pregnancy: outstanding issues. Reprod Fertility Dev 13: 615-22, 2001. [PMID: 11999313]
  244. Thoms V, Licastro F, **Masliah E**. Locally reduced levels of acidic FGF lead to decreased expression of 28-kDa calbindin and contribute to the selective vulnerability of the neurons in the entorhinal cortex in Alzheimer's disease. Neuropathology 21: 203-11, 2001. [PMID: 11666017]
  245. **Masliah E**, Alford M, Galasko D, Salmon D, Hansen LA, Good PF, Perl DP, Thal L. Cholinergic deficits in the brains of patients with parkinsonism-dementia complex of Guam. Neuroreport 12:3901-3, 2001. [PMID: 11742207]
  246. Rockenstein E, Mallory M, Mante M, Sisk A, **Masliah E**. Early formation of mature amyloid- $\beta$  protein deposits in a mutant APP transgenic model depends on levels of A $\beta$  (1-42). J Neurosci Res 66:573-82, 2001. [PMID: 11746377]
  247. Xia Y, Saitoh T, Kang D, Tanaka S, Chen X, Hashimoto M, Hsu L, Conrad C, Sundsmo M, Yoshimoto M, Thal LJ, Katzman R, **Masliah E**. Characterization of the human NACP/ $\alpha$ -synuclein gene: Genomic structure, transcription start site, promoter region and polymorphisms. J Alz Dis 3: 485-94, 2001. [PMID: 12214035]
- 2002**
248. Hashimoto M, Hsu Leigh, Rockenstein E, Takenouchi T, Mallory M, **Masliah E**.  $\alpha$ -Synuclein protects against oxidative stress via inactivation of the c-Jun N-terminal kinase stress-signaling pathway in neuronal cells. J Biol Chem 277:11465-72, 2002. [PMID: 11790792]
  249. Rockenstein E, Mallory M, Mante M, Alford M, Windisch M, Moessler H, **Masliah E**. Effects of Cerebrolysin on amyloid-beta deposition in a transgenic model of Alzheimer's disease. J Neural Transm Suppl 62:327-36, 2002. [PMID: 12456076]
  250. Langford TD, Letendre S, Mallory M, Hansen L, Archibald S, Jernigan T, **Masliah E**, the HNRC Group. Severe, demyelinating leukoencephalopathy in AIDS patients failing antiretroviral therapy. AIDS 16:1-12, 2002. [PMID: 11953468]
  251. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, **Masliah E**, Goldberg MS, Shen J, Takio K, Iwatsubo T.  $\alpha$ -Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4:160-4, 2002. [PMID: 11813001]
  252. Veinbergs I, Everson A, Sagara Y, **Masliah E**. Neurotoxic effects of apolipoprotein E4 are mediated via dysregulation of calcium homeostasis. J Neurosci Res 67:379-87, 2002. [PMID: 11813243]

253. Rose SC, Behling C, Roberts AC, Nelson TR, Kinney TB, **Masliah E**, Hassanein TI. Main portal vein access in transjugular intrahepatic portosystemic shunt procedures: use of three-dimensional ultrasound to ensure safety. *J Vasc Interv Radio* 13:267-73, 2002. [PMID: 11875086]
254. Overstreet K, Benirschke K, Scioscia A, **Masliah E**. Congenital nephrosis of the Finnish type: overview of placental pathology and literature review. *Pediatr Dev Pathol* 5:179-83, 2002. [PMID: 11910513]
255. Woulfe JM, Duke R, Middeldorp JM, Stevens S, Vervoort M, Hashimoto M, **Masliah E**, Chan P, Di Monte DA, Langston JW, Petzinger G, Hoogendoorn H, Munoz DG. Absence of elevated anti- $\alpha$ -synuclein and anti-EBV latent membrane protein antibodies in PD. *Neurology* 58:1435-6, 2002. [PMID: 12011302]
256. Garden GA, Budd SL, Tsai E, Hanson L, Kaul M, D'Emilia DM, Friedlander RM, Yuan J, **Masliah E**, Lipton SA. Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *J Neurosci* 22:4015-24, 2002. [PMID: 12019321]
257. Langford TD, Sanders VJ, Mallory M, Kaul M, **Masliah E**. Expression of stromal cell-derived factor 1  $\alpha$  protein in HIV encephalitis. *J Neuroimmunol* 127:115-26, 2002. [PMID: 12044982]
258. Hashimoto M, Sagara Y, Langford TD, Everall IP, Mallory M, Everson A, Digicaylioglu M, **Masliah E**. Fibroblast growth factor 1 regulates signaling via the GSK3 $\beta$  pathway: implications for neuroprotection. *J Biol Chem* 277:32985-991, 2002. [PMID: 12095987]
259. Rockenstein E, Mallory M, Hashimoto M, Song D, Shultz C, Lang I, **Masliah E**. Differential neuropathological alterations in transgenic mice expressing  $\alpha$ -synuclein from the PDGF-B and Thy-1 promoters. *J Neurosci Res* 68:568-78, 2002. [PMID: 12111846]
260. Takehashi M, Tanaka S, **Masliah E**. Association of monoamine oxidase a gene polymorphism with Alzheimer's disease and Lewy body variant. *Neurosci Lett* 327:79-82, 2002. [PMID: 12098640]
261. Tanaka S, Takehashi M, Matoh N, Iida S, Suzuki T, Futaki S, Hamada H, **Masliah E**, Ueda K. Generation of reactive oxygen species and activation of NF- $\kappa$ B by non- $\alpha$  component of Alzheimer's disease amyloid. *J Neurochem* 82:305-15, 2002. [PMID: 12124431]
262. Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, Quigg RJ, **Masliah E**. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci USA* 99:10837-42, 2002. [PMID: 12119423]
263. Windisch M, Hutter-Paier B, Rockenstein E, Hashimoto M, Mallory M, **Masliah E**. Development of a new treatment for Alzheimer's disease and Parkinson's disease using anti-aggregatory beta-synuclein-derived peptides. *J Mol Neurosci* 19:63-9, 2002. [PMID: 12212795]
264. Chismar JD, Mondala T, Fox HS, Roberts E, Langford TD, **Masliah E**, Salomon DR, Head SR. Analysis of result variability from high-density oligonucleotide arrays comparing same-species and cross-species hybridizations. *Biotechniques* 33:516-8, 2002. [PMID: 12238761]
265. **Masliah E**, Hashimoto M. Development of new treatments for Parkinson's disease in transgenic animal models: A role for  $\beta$ -synuclein. *NeuroToxicology* 23:461-8, 2002. [PMID: 12428718]
266. Langford TD, **Masliah E**. Role of trophic factors on neuroimmunity in neurodegenerative infectious diseases. *J NeuroViro* 8:625-38, 2002. [PMID: 12476355]
267. Wyss-Coray T, Yan F, Hsui-Ti Lin A, Lambris JD, Alexander JJ, Quigg RJ, **Masliah E**. Prominent neurodegeneration and increased plaque formation in complemented-inhibited Alzheimer's mice. *Proc Natl Acad Sci USA* 99:10837-42, 2002. [PMID: 12119423]

268. Overstreet K, Costanza C, Behling C, Hassanin T, **Masliah E**. Fatal progressive hepatic necrosis associated with lamotrigine treatment: A case report and literature review. Dig Dis Sci 47:1921-5, 2002. [PMID: 12353830]
269. Morgello S, Gelman BB, Kozlowski PB, Vinters HV, **Masliah E**, Cornford M, Cavert W, Marra C, Grant I, Singer EJ. The National NeuroAIDS Tissue Consortium: a new paradigm in brain banking with an emphasis on infectious disease. Neuropathol Appl Neurobiol 27:326-35, 2002. [PMID: 11532163]
270. Cherner M, **Masliah E**, Ellis RJ, Marcotte TD, Moore DJ, Grant I, Heaton RK. Neurocognitive dysfunction predicts postmortem findings in HIV encephalitis. Neurology 26:59:1563-67, 2002. [PMID: 12451198]
271. Tietz A, Oron L, **Masliah E**, Michaelson DM. The effects of apolipoprotein E genotype on brain lipid metabolism. J Alzheimer Dis
273. Everall IP, Bell C, Mallory M, Langford D, Adame A, Rockenstein E, **Masliah E**. Lithium ameliorates HIV-gp120-mediated neurotoxicity. Mol Cell Neurosci 21:493-501, 2002. [PMID: 12498789]
274. Ho GJ, Hansen LA, Alford MF, Foster K, Salmon DP, Galasko D, Thal LJ, **Masliah E**. Age at onset is associated with disease severity in Lewy body variant and Alzheimer's disease. Neuroreport 13:1825-28, 2002. [PMID: 12395133]
275. **Masliah E**, Hansen LA, Rockenstein E, Hashimoto M. Progress in the development of new treatments for combined Alzheimer's and Parkinson's disease. Drug Development Research 56:282-292, 2002.
276. Van Uden E, Mallory M, Veinbergs I, Alford M, Rockenstein E, **Masliah E**. Increased extracellular amyloid deposition in human amyloid precursor protein transgenic mice deficient in receptor-associated protein. J Neurosci 22:9298-9304, 2002. [PMID: 12417655]
277. Triaboschi P, Hansen LA, Alford M, Merdes A, **Masliah E**, Thal LJ, Corey-Bloom J. Early and widespread cholinergic losses differentiate dementia with Lewy bodies from Alzheimer disease. Arch Gen Psychiatry 59:946-51, 2002. [PMID: 12365882]
278. Buttini M, Yu GQ, Shockley K, Huang Y, Jones B, **Masliah E**, Mallory M, Yeo T, Longo FM, Mucke L. Modulation of Alzheimer-like synaptic and cholinergic deficits in transgenic mice by human apolipoprotein E depends on isoform, aging, and overexpression of amyloid beta peptides but not on plaque formation. J Neurosci 22:10539-48, 2002. [PMID: 12486146]

## 2003

279. Gonzalez R, Heaton RK, Moore DJ, Letendre S, Ellis RJ, Wolfson T, Marcotte T, Cherner M, Rippeth J, Grant I, Atkinson JH, McCutchan JA, Marcotte TD, Wallace MR, Schrier R, Jernigan T, Hesselink J, **Masliah E**, Masys DR, Frybarger M, Abramson I, Deutsch R. Computerized reaction time battery versus a traditional neuropsychological battery: detecting HIV-related impairments. J Int Neuropsychol Soc 9:64-71, 2003.
280. Marr RA, Rockenstein E, Mukherjee A, Kindy MS, Hersh LB, Gage FH, Verma IM, **Masliah E**. Nepriysin gene transfer reduces human amyloid pathology in transgenic mice. J Neurosci 23:1992-6, 2003.
281. Langford TD, Letendre SL, Larrea GJ, **Masliah E**. Changing patterns in the neuropathogenesis of HIV during the HAART era. Brain Pathol 13:195-210, 2003.

282. Kennedy BP, Zielger MG, Alford M, Hansen LA, Thal LJ, **Masliah E**. Early and persistent alterations in prefrontal cortex MAO A and B in Alzheimer's disease. J Neural Trans 110:789-801, 2003.
283. Hashimoto M, Takenouchi T, Rockenstein E, **Masliah E**. alpha-Synuclein up-regulates expression of caveolin-1 and down-regulates extracellular signal-regulated kinase activity in B103 neuroblastoma cells: role in the pathogenesis of Parkinson's disease. J Neurochemistry 85:1468-79, 2003.
284. Bannykh SI, Emery SC, Gerber JK, Jones KL, Benirschke K, **Masliah E**. Aberrant *Pax1* and *Pax9* expression in Jarcho-Levin syndrome: Report of two Caucasian siblings and literature review. Am J Med Genetics 120A:241-46, 2003.
285. Hashimoto M, Rockenstein E, **Masliah E**. Transgenic models of  $\alpha$ -synuclein pathology: past, present and future. NY Acad Sci 991:171-88, 2003.
286. Samland H, Huitron-Resendiz S, **Masliah E**, Criado J, Henriksen SJ, Campbell IL. Profound increase in sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. J Neurosci Res 73:176-87, 2003.
287. da Costa A, **Masliah E**, Checler F. Beta-synuclein displays an antiapoptotic p53-dependent phenotype and protects neurons from 6-hydroxydopamine-induced caspase 3 activation: cross-talk with alpha-synuclein and implication for Parkinson's disease. J Biol Chem 278:37330-35, 2003.
288. Palop JJ, Jones B, Kekoni L, Chin J, Yu GQ, Raber J, **Masliah E**, Mucke L. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc Natl Acad Sci USA 100:9572-77, 2003.
289. Licastro F, Grimaldi LME, Bonafe M, Martina C, Olivieri F, Cavallone L, Giovannetti S, **Masliah E**, Franceschi C. Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. Neurobiol of Aging 24:921-6, 2003.
290. **Masliah E**, Alford M, Adame A, Rockenstein E, Galasko D, Salmon D, Hansen LA, Thal LJ. A $\beta$ 1-42 promotes cholinergic sprouting in patients with AD and Lewy body variant of AD. Neurology 61:206-11, 2003.
291. Hashimoto M, **Masliah E**. Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. Neurochem Res 28:1743-56, 2003.
292. Hashimoto M, Rockenstein E, Crews L, **Masliah E**. The role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's disease. Neuromolecular Med 4:21-36, 2003.
293. Harris FM, Brecht WJ, Xu Q, Tesser I, Kekoni L, Wyss-Coray T, Fish JD, **Masliah E**, Hopkins PC, Searce-Levie K, Weisgraber KH, Mucke L, Mahley RW, Huang Y. Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. Proc Natl Acad Sci USA 100:10966-71, 2003.
294. Langford D, Adame A, Grigorian A, Grant I, McCutchan JA, Ellis R, Marcotte TD, **Masliah E**, the HNRC group. Patterns of selective neuronal damage in methamphetamine-user AIDS patients. J Acquir Immune Defic Syndr 34:467-74, 2003.
295. Langford TD, **Masliah E**. The emerging role of infectious pathogens in neurodegenerative diseases. Exp Neurology 184:553-5, 2003.

296. Matoh N, Tanaka S, Takehashi M, Banasik M, Stedeford T, **Masliah E**, Suzuki S, Nishimura Y, Ueda K. Overexpression of CYP2D6 attenuates the toxicity of MPP in actively dividing and differentiated PC12 cells. Gene Expression 11:117-24, 2003.
297. Rockenstein E, Anthony A, Mante M, Moessler H, Windisch M, **Masliah E**. The neuroprotective effects of Cerebrolysin™ in a transgenic model of Alzheimer's disease are associated with improved behavioral performance. J Neurotransm 28:1313-17, 2003.
298. Lu DC, Shaked GM, **Masliah E**, Bredesen DE Koo EH. Amyloid beta protein toxicity mediated by the formation of amyloid-beta protein precursor complexes. Ann Neurol 54:781-9, 2003.
299. Brionne TC, **Masliah E**, Wyss-Coray T. Loss of TGF- $\beta$ 1 leads to increased neuronal cell death and microgliosis in mouse brain. Neuron 40:1133-45, 2003.

## 2004

300. Marr RA, Guan H, Rockenstein E, Kindy M, Gage FH, Verma I, **Masliah E**, Hersch LB. Neprilysin regulates amyloid Beta peptide levels. J Molec Neurosci 22:5-11, 2004.
301. **Masliah E**, Wahl C. Umbilical cord stricture in a 21-week fetus. J Perinatol 24:48-9, 2004.
302. Solforosi L, Criado JR, McGavern DB, Wirz S, Sanchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, **Masliah E**, Gilden D, Oldstone MB, Conti B, Williamson RA. Cross-linking cellular prion protein triggers neuronal apoptosis in vivo. Science 303:1514-6, 2004.
303. Bannykh GI, Benirschke K, **Masliah E**, Bannykh SI. Failure of prosencephalic unfolding and neuronal migration in acardia. Acta Neuropathol 107:319-30, 2004
304. Emery SC, Vaux KK, Pretorius D, **Masliah E**, Benirschke K. Acardiac twin with externalized intestine adherent to placenta: unusual manifestation of omphalocele. Pediatr Dev Pathol 7:81-5, 2004.
305. Archibald SL, **Masliah E**, Fennema-Notestine C, Marcotte TD, Ellis RJ, McCutchan JA, Heaton RK, Grant I, Mallory M, Miller A, Jernigan TL, the HNRC Group. Correlation of in vivo neuroimaging abnormalities with postmortem human immunodeficiency virus encephalitis and dendritic loss. Arch Neurology 61:369-76, 2004.
306. Song DD, Shults CW, Sisk A, Rockenstein E, **Masliah E**. Enhanced substantia nigra mitochondrial pathology in human  $\alpha$ -synuclein transgenic mice after treatment with MPTP. Exp Neurol 186:158-72, 2004.
307. Hashimoto M, Bar-On P, Ho G, Takenouchi T, Rockenstein E, Crews L, **Masliah E**.  $\beta$ -Synuclein regulates Akt activity in neuronal cells: a possible mechanism for neuroprotection in Parkinson's disease. J Biol Chem 279:23622-29, 2004.
308. Klucken J, Shin Y, **Masliah E**, Hyman BT, McClean PJ. Hsp70 reduces on alpha-synuclein aggregation and toxicity. J Biol Chem 279:25497-502, 2004.
309. Kennedy BP, Bottiglieri T, Arning E, Ziegler MG, Hansen LA, **Masliah E**. Elevated S-adenosylhomocysteine in Alzheimer brain: influence on methyltransferases and cognitive function. J Neural Transm 111:547-67, 2004.
310. Fuller RA, Westmoreland SV, Ratai E, Greco JB, Kim JP, Lentz MR, He J, Seghal PK, **Masliah E**, Lackner AA, González RG. A prospective longitudinal MR spectroscopy study of the SIV/Macaque model of neuroAIDS. BMC Neuroscience 5:10, 2004.



311. Tiraboschi P, Sabbagh MN, Hansen LA, Salmon DP, Olichney JM, Gamst A, **Masliah E**, Thal LJ, Corey-Bloom J. Alzheimer's disease without neocortical neurofibrillary tangles: "A second look". Neurology 62:1141-7, 2004.
312. Carlson KA, Limoges J, Pohlman GD, Poluektova L, Langford D, **Masliah E**, Ikso T, Gendelman HE. OTK18 expression in brain mononuclear phagocytes parallels the severity of Hiv-1 encephalitis. J NeuroImmunol 150:186-98, 2004.
313. Chin J, Palop JJ, Yu G, **Masliah E**, Mucke L. Fyn kinase modulates synaptotoxicity, but not aberrant sprouting, in transgenic mice with high A $\beta$ 1-42 levels. J Neurosci 24:4692-7, 2004.
314. Greco JB, Westmoreland SV, Sakaie K, He J, Aminpour S, Lee PL, Cheng LL, Seghal PK, **Masliah E**, Lackner AA, Gonzalez RG. In vivo 1H MRS of brain injury and repair during acute SIV infection in the macaque model of neuroAIDS. Mag Resonance in Med 51:10108-14, 2004.
315. Yao D, Gu Z, Nakamura T, Shi ZQ, Ma Y, Gaston B, Palmer LA, Rockenstein EM, Zhang Z, **Masliah E**, Uehara T, Lipton SA. Nitrosative stress linked to sporadic Parkinson's disease: S-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. Proc Natl Acad Sci USA 101:10810-4, 2004.
316. Licastro F, Veglia F, Grimaldi LM, **Masliah E**. A polymorphism of the interleukin-1 beta gene at position +3953 influences progression and neuro-pathological hallmarks of Alzheimer's disease. Neurobiol Aging 25:1017-22, 2004.
317. Takehashi M, Alioto T, Stedeford T, **Masliah E**, Tanaka S, Ueda K. Septin 3 gene polymorphism in Alzheimer's disease. Gene Expr 11:263-70, 2004.
318. Tiraboschi P, Hansen LA, Alford M, **Masliah E**, Thal LJ, Corey-Bloom J. Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. Neurology 62:1977-83, 2004.
319. Licastro F, Chiappelli M, Thal LJ, **Masliah E**. Alpha-1-Antichymotrypsin polymorphism in the gene promoter region affects survival and synapsis loss in Alzheimer's disease. Arch Gerontol Geriatr 38:243-251, 2004.
320. Hamilton JM, Salmon DP, Galasko D, Delis DC, Hansen LA, **Masliah E**, Thomas RG, Thal LJ. A comparison of episodic memory deficits in neuropathologically-confirmed Dementia with Lewy bodies and Alzheimers disease. J Int Neuropsychol Soc 10:689-97, 2004.
321. Hockett P, Emery SC, Hansen L, **Masliah E**. Evidence of oxidative stress in the brains of fetuses with CNS anomalies and islet cell hyperplasia. Pediat Dev Pathol 7:370-9, 2004.
322. Crews L, Wyss-Coray T, **Masliah E**. Insights into the pathogenesis of hydrocephalus from transgenic and experimental animal models. Brain Pathol 14:312-6, 2004.
323. Hashimoto M, Rockenstein E, Mante M, Crews L, Bar-On P, Gage F, Marr R, **Masliah E**. An anti-aggregation gene therapy strategy for Lewy body disease utilizing  $\beta$ -synuclein lentivirus in a transgenic model. Gene Therapy 24:9434-40, 2004.
324. Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, **Masliah E**, Levine MS, Chesselet MF. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. J Neurosci 24:9434-40, 2004.
325. Roberts ES, **Masliah E**, Fox HS. CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). J Neuropathol Exp Neurol 63:1255-64, 2004.

326. Belichenko PV, **Masliah E**, Kleschevnikov AM, Villar AJ, Epstein CJ, Salehi A, Mobley WC. Synaptic structural abnormalities in the Ts65Dn mouse model of Down Syndrome. J Comp Neurol 480:281-98, 2004.
327. Langford D, Grigorian A, Hurford R, Adame A, Ellis RJ, Hansen L, **Masliah E**. Altered P-glycoprotein expression in AIDS patients with HIV encephalitis. J Neuropathol Exp Neurol 63:1038-47, 2004.
328. Achim CL, **Masliah E**, Schindelar J, Avramut M. Immunophilin expression in the HIV-infected brain. J Neuroimmunol 157:126-32, 2004.
329. **Masliah E**, Roberts ES, Langford D, Everall I, Crews L, Adame A, Rockenstein E, Fox HS. Patterns of gene dysregulation in the frontal cortex of patients with HIV encephalitis. J Neuroimmunol 157:163-75, 2004.
330. Winner B, Lie DC, Rockenstein E, Aigner R, Aigner L, **Masliah E**, Kuhn HG, Winkler J. Human wild-type alpha-synuclein impairs neurogenesis. J Neuropathol Exp Neurol 63:1155-66, 2004.
331. Pham TT, Benirschke K, **Masliah E**, Stocker JT, Yi ES. Congenital pulmonary airway malformation (congenital cystic adenomatoid malformation) with multiple extrapulmonary anomalies: autopsy report of a fetus at 19 weeks of gestation. Pediatr Dev Pathol 7:661-6, 2004.
332. Hashimoto M, Kawahara K, Bar-On P, Rockenstein E, Crews L, **Masliah E**. The Role of alpha-Synuclein Assembly and Metabolism in the Pathogenesis of Lewy Body Disease. J Mol Neurosci 24:343-52, 2004.
333. Langford D, Grigorian A, Hurford R, Adame A, Crews L, **Masliah E**. The role of mitochondrial alterations in the combined toxic effects of human immunodeficiency virus Tat protein and methamphetamine on calbindin positive-neurons. J Neurovirol 10:327-37, 2004.

## 2005

334. Zhou X, Long JM, Geyer MA, **Masliah E**, Kelsoe JR, Wynshaw-Boris A, Chien KR. Reduced expression of the Sp4 gene in mice causes deficits in sensorimotor gating and memory associated with hippocampal vacuolization. Mol Psychiatry 10:393-406, 2005.
335. Snyder H, Mensah K, Hsu C, Hashimoto M, Surgucheva IG, Festoff B, Surguchov A, **Masliah E**, Matouschek A, Wolozin B. beta-synuclein reduces proteasomal inhibition by alpha-synuclein but not gamma-synuclein. J Biol Chem 280:7562-9, 2005.
336. **Masliah E**, Hansen L, Adame A, Crews L, Bard F, Lee C, Seubert P, Games D, Kirby L, Schenk D. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. Neurology 64:129-31, 2005.
337. Rockenstein E, Adame A, Mante M, Larrea G, Crews L, Windisch M, Moessler H, **Masliah E**. Amelioration of the cerebrovascular amyloidosis in a transgenic model of Alzheimer's disease with the neurotrophic compound Cerebrolysintrade mark. J Neural Transm 112:269-82, 2005.
338. Ho GJ, Hashimoto M, Adame A, Izu M, Alford MF, Thal LJ, Hansen LA, **Masliah E**. Altered p59(Fyn) kinase expression accompanies disease progression in Alzheimer's disease: implications for its functional role. Neurobiol Aging 26:625-35, 2005.
339. Langford D, Hurford R, Hashimoto M, Digicaylioglu M, **Masliah E**. Signalling crosstalk in FGF2-mediated protection of endothelial cells from HIV-gp120. BMC Neurosci 6:8, 2005.

340. Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, **Masliah E**, Williams DS, Goldstein LS. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science 307:1282-8, 2005.
341. Rockenstein E, Schwach G, Ingolic E, Adame A, Crews L, Mante M, Pfragner R, Schreiner E, Windisch M, **Masliah E**. Lysosomal pathology associated with alpha-synuclein accumulation in transgenic models using an eGFP fusion protein. J Neurosci Res 80:247-59, 2005.
342. Green DA, **Masliah E**, Vinters HV, Beizai P, Moore DJ, Achim CL. Brain deposition of beta-amyloid is a common pathologic feature in HIV positive patients. AIDS 19:407-411, 2005.
343. Lentz MR, Kim JP, Westmoreland SV, Greco JB, Fuller RA, Ratai EM, He J, Sehgal PK, Halpern EF, Lackner AA, **Masliah E**, Gonzalez RG. Quantitative Neuropathologic Correlates of Changes in Ratio of N-acetylaspartate to Creatine in Macaque Brain. Radiology 235:461-8, 2005.
344. Kim JP, Lentz MR, Westmoreland SV, Greco JB, Ratai EM, Halpern E, Lackner AA, **Masliah E**, Gonzalez RG. Relationships between astrogliosis and 1H MR spectroscopic measures of brain choline/creatine and myo-inositol/creatine in a primate model. Am J Neuroradiol 26:752-9, 2005.
345. Ho GJ, Drego R, Hakimian E, **Masliah E**. Mechanisms of cell signaling and inflammation in Alzheimer's disease. Curr Drug Targets Inflamm Allergy 4:247-56, 2005.
346. Tsuboi K, Grzesiak JJ, Bouvet M, Hashimoto M, **Masliah E**, Shults CW. Alpha-synuclein overexpression in oligodendrocytic cells results in impaired adhesion to fibronectin and cell death. Mol Cell Neurosci 29:259-68, 2005.
347. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, **Masliah E**, Oldstone M. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 308:1435-9, 2005.
348. **Masliah E**, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Lee M, Chilcote T, Games D, Schenk D. Effects of  $\alpha$ -synuclein immunizations in a mouse model of Parkinson's disease. Neuron 46:857-68, 2005.
349. Chevallier NL, Soriano S, Kang DE, **Masliah E**, Hu G, Koo EH. Perturbed Neurogenesis in the Adult Hippocampus Associated with Presenilin-1 A246E Mutation. Am J Pathol 167:151-9, 2005.
350. Rockenstein E, Mante M, Alford M, Adame A, Crews L, Hashimoto M, Maya S, Esposito L, Mucke L, **Masliah E**. High  $\beta$ -secretase activity elicits neurodegeneration in transgenic mice and despite reductions in amyloid- $\beta$  levels: implications for the treatment of Alzheimer's disease. J. Biol. Chem 280:32957-67, 2005.
351. Wu C, Thal L, Pizzo D, Hansen L, **Masliah E**, Geula C. Apoptotic Signals within the Basal Forebrain Cholinergic Neurons in Alzheimer's Disease. Exp Neuro 195:484-496, 2005.
352. Williams K, Westmoreland S, Greco J, Ratai E, Lentz M, Kim WK, Fuller R, Kim JP, Autissier P, Sehgal P, Shinazi R, Bischofberger N, Piatik M, Lifson J, **Masliah E**, Gonzalez RG. Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. J Clin Invest 115:2534-2545, 2005.
353. Singer O, Marr R, Rockenstein E, Crews L, Gage F, Verma I, **Masliah E**. Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. Nature Neuroscience 8:1343-9, 2005.

354. Buttini M, **Masliah E**, Barbour R, Grajeda H, Motter R, Johnson-Wood K, Khan K, Seubert P, Freedman S, Schenk D, Games D.  $\beta$ -Amyloid Immunotherapy Prevents Synaptic Degeneration in a Mouse Model of Alzheimer's Disease. J Neurosci 25:9096-101, 2005.
  355. Chin J, Palop JJ, Puolivali J, Massaro C, Bien-Ly N, Gersetein H, Searce-Levie K, **Masliah E**, Mucke L. Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. J Neurosci 25:9694-703, 2005.
  356. Everall I, Salaria S, Roberts E, Corbeil J, Sasik R, Fox H, Grant I, **Masliah E**, HNRC Group. Methamphetamine stimulates interferon inducible genes in HIV infected brain. J Neuroimmunol 170:158-71, 2005.
  357. Everall IP, Hansen LA, **Masliah E**. The shifting patterns of HIV encephalitis neuropathology. Neurotox Res 8:51-62, 2005.
  358. Bellizzi M, Lu SM, **Masliah E**, Gelbard H. Synaptic activity becomes excitotoxic in neurons exposed to elevated levels of platelet-activating factor. J Clin Invest 115:3185-92, 2005.
  359. Gilman S, May SJ, Shults CW, Tanner CM, Kukull W, Lee VM, **Masliah E**, Low P, Sandroni P, Trojanowski JQ, Ozelius L, Foroud T; and The North American Multiple System Atrophy Study Group. The North American multiple system atrophy study group. J Neural Transm 112:1687-94, 2005. [PMID: 16284910]
  360. Shults C, Rockenstein E, Crews L, Adame A, Mante M, Larrea G, Hashimoto M, Song D, Iwatsubo T, Tsuboi K, **Masliah E**. Neurological and neurodegenerative alterations in a transgenic mouse model expressing human alpha-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. J Neurosci 25:10689-99, 2005.
  361. Langford TD, Marquie-Beck J, de Almeida S, Lazzaretto D, Letendre S, Grant I, McCutchan A, **Masliah E**, Ellis R. Relationship of antiretroviral treatment during life to post-mortem brain tissue viral load in HIV-infected patients. J NeuroVirology 12:100-7, 2005.
- 2006**
362. Peterson MR, Emery SC, Yung GL, **Masliah E**, Yi ES. Epstein-Barr Virus-Associated Posttransplantation Lymphoproliferative Disorder Following Lung Transplantation Is More Commonly of Host Origin. Arch Pathol Lab Med 130:176-180, 2006.
  363. Webster B, Hansen L, Adame A, Crews L, Torrance M, Thal L, **Masliah E**. Astroglial activation of ERK in early stages of Alzheimer's Disease. J Neuropath Exp Neurol 65:142-51, 2006.
  364. Rockenstein E, Torrance M, Mante M, Adame A, Paulino A, Rose J, Crews L, Moessler H, **Masliah E**. Cerebrolysin decreases amyloid-beta production by regulating amyloid protein precursor maturation in a transgenic model of Alzheimer's disease. J Neurosci Res 83:1252-61, 2006.
  365. Moore D, **Masliah E**, Rippeth J, Gonzalez R, Carey C, Cherner M, Ellis R, Achim C, Marcotte T, Heaton R, Grant I, HNRC Group. Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. AIDS 20:879-887, 2006.
  366. Esposito L, Raber J, Kekoni L, Yan F, Yu GQ, Bien-Ly N, Puolivali J, Searce-Levie K, **Masliah E**, Mucke L. Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. J Neurosci 26:5167-79, 2006.

367. Waragai M, Wei J, Fujita M, Nakai M, Ho GJ, **Masliah E**, Akatsu H, Yamada T, Hashimoto M. Increased level of DJ-1 in the cerebrospinal fluids of sporadic Parkinson's disease. Biochem Biophys Res Commun 345:967-72, 2006.
368. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, **Masliah E**, Nomura Y, Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441:513-7, 2006.
369. Trifilo MJ, Yajima T, Gu Y, Dalton N, Peterson KL, Race RE, Meade-White K, Portis JL, **Masliah E**, Knowlton KU, Chesebro B, Oldstone MB. Prion-induced amyloid heart disease with high blood infectivity in transgenic mice. Science 313:94-7, 2006.
370. Bar-On P, Rockenstein E, Adame A, Ho G, Hashimoto M, **Masliah E**. Effects of cholesterol lowering compound methyl- $\beta$ -cyclodextrin in models of  $\alpha$ -synucleopathy. J. Neurochem 98:1032-45, 2006.
371. Berman JW, Carson MJ, Chang L, Cox BM, Fox HS, Gonzalez RG, Hanson GR, Hauser KF, Ho WZ, Hong JS, Major EO, Maragos WF, **Masliah E**, McArthur JC, Miller DB, Nath A, O'Callaghan JP, Persidsky Y, Power C, Rogers TJ, Royal W 3rd. NeuroAIDS, drug abuse, and inflammation: building collaborative research activities. J Neuroimmune Pharmacol 1:351-99, 2006.
372. **Masliah E**, Crews L, Hansen L. Synaptic remodeling during aging and in Alzheimer's disease. J Alz Dis 9:91-99, 2006.
373. Fleming S, Salcedo J, Hutson C, Rockenstein E, **Masliah E**, Levine M, Chesselet MF. Behavioral effects of dopaminergic agonists in transgenic mice overexpressing human wildtype alpha-synuclein. Neuroscience 142:1245-53, 2006.
374. Patton RL, Kalback WM, Esh CL, Kokjohn TA, Van Vickle GD, Luehrs DC, Kuo YM, Lopez J, Brune D, Ferrer I, **Masliah E**, Newel AJ, Beach TG, Castano EM, Roher AE. Amyloid- $\beta$  Peptide Remnants in AN-1792-Immunized Alzheimer's Disease Patients: A Biochemical Analysis. Am J Pathol 169:1048-63, 2006.
375. Bakhtar O, Benirschke K, **Masliah E**. Sirenomelia of an intracytoplasmic sperm injection conceptus: a case report and review of mechanism. Pediatr Dev Pathol 9:245-53, 2006.
376. Mandal PK, Pettegrew JW, **Masliah E**, Hamilton RL, Mandal R. Interaction between Abeta Peptide and alpha Synuclein: Molecular Mechanisms in Overlapping Pathology of Alzheimer's and Parkinson's in Dementia with Lewy Body Disease. Neurochem Res 31:1153-62, 2006.
377. Letendre SL, Woods SP, Ellis RJ, Atkinson JH, **Masliah E**, van den Brande G, Durelle J, Grant I, Everall I, the HNRC Group. Lithium improves HIV-associated neurocognitive impairment. AIDS 20:1885-8, 2006.
378. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, **Masliah E**, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314:130-3, 2006.
379. Chana G, **Masliah E**, Langford D, Adame A, Crews L, Grant I, Cherner M, Lazzaretto D, Heaton R, Everall P, HNRC Group. Cognitive deficits and degeneration of interneurons in HIV+ methamphetamine users. Neurology 67:1486-9, 2006.
380. Tesseur I, Zou K, Esposito L, Bard F, Berber E, Can JV, Lin AH, Crews L, Tremblay P, Mathews P, Mucke L, **Masliah E**, Wyss-Coray T. Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. J Clin Invest 116:3060-3069, 2006.

381. Fujita M, Wei J, Nakai M, **Masliah E**, Hashimoto M. Chaperone and anti-chaperone: two-faced synuclein as stimulator of synaptic evolution. Neuropathology 26:383-92, 2006.
  382. Luo J, Lin AH, **Masliah E**, Wyss-Coray T. Bioluminescence imaging of Smad signaling in living mice shows correlation with excitotoxic neurodegeneration. Proc Natl Acad Sci U S A 103:18326-31, 2006.
  383. Everall IP, Salari S, Atkinson JH, Young C, Corbeil J, Grant I, **Masliah E**, HNRC (HIV Neurobehavioral Research Center). Diminished somatostatin gene expression in individuals with HIV and major depressive disorder. Neurology 67:1867-9, 2006.
- 2007**
384. Rockenstein E, Mante M, Adame A, Crews L, Moessler H, **Masliah E**. Effects of Cerebrolysintrade mark on neurogenesis in an APP transgenic model of Alzheimer's disease. Acta Neuropathol (Berl) 113:265-75, 2007.
  385. Zhou X, Qyang Y, Kelsoe JR, **Masliah E**, Geyer MA. Impaired postnatal development of hippocampal dentate gyrus in Sp4 null mutant mice. Genes Brain Behav 6:269-76 2007.
  386. Ruiz PJ, **Masliah E**, Doherty TA, Quach A, Firestein GS. Cardiac death in a patient with adult onset Still's disease treated with the IL-1 receptor inhibitor anakinra. Ann Rheum Dis 66:422-3, 2007.
  387. Ellis R, Langford D, **Masliah E**. HIV and antiretroviral therapy in the brain: neuronal injury and repair. Nat Rev Neurosci 8:33-44, 2007.
  388. Spencer B, Crews L, **Masliah E**. Climbing the scaffolds of Parkinson's disease pathogenesis. Neuron 53:469-70, 2007.
  389. Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, **Masliah E**. Neuroprotective effects of regulators of the glycogen synthase kinase-3 $\beta$  signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J Neurosci 27:1981-91, 2007.
  390. Lippa CF, Duda JE, Grossman M, Hurtig HI, Aarsland D, Boeve BF, Brooks DJ, Dickson DW, Dubois B, Emre M, Fahn S, Farmer JM, Galasko D, Galvin JE, Goetz CG, Growdon JH, Gwinn-Hardy KA, Hardy J, Heutink P, Iwatsubo T, Kosaka K, Lee VM, Leverenz JB, **Masliah E**, McKeith IG, Nussbaum RL, Olanow CW, Ravina BM, Singleton AB, Tanner CM, Trojanowski JQ, Wszolek ZK, DLB/PDD Working Group. DLB and PDD boundary issues: diagnosis, treatment, molecular pathology, and biomarkers. Neurology 68:812-9, 2007.
  391. Tsigelny IF, Bar-On P, Sharikov Y, Crews L, Hashimoto M, Miller MA, Keller SH, Platoshyn O, Yuan JX, **Masliah E**. Dynamics of alpha-synuclein aggregation and inhibition of pore-like oligomer development by beta-synuclein. FEBS J 274:1862-77, 2007.
  392. Cherner M, Cysique L, Heaton RK, Marcotte TD, Ellis RJ, **Masliah E**, Grant I; HNRC Group. Neuropathologic confirmation of definitional criteria for human immunodeficiency virus-associated neurocognitive disorders. J Neurovirol 13:23-8, 2007.
  393. Duffy BM, Warner LR, Hou ST, Jiang SX, Gomez-Isla T, Leenhouts KM, Oxford JT, Feany MB, **Masliah E**, Rohn TT. Calpain-Cleavage of {alpha}-Synuclein: Connecting Proteolytic Processing to Disease-Linked Aggregation. Am J Pathol 170:1725-38, 2007.

394. Letendre S, Paulino AD, Rockenstein E, Adame A, Crews L, Cherner M, Heaton R, Ellis R, Everall IP, Grant I, **Masliah E**, HIV Neurobehavioral Research Center Group. Pathogenesis of Hepatitis C Virus Coinfection in the Brains of Patients Infected with HIV. J Infect Dis 196:361-70, 2007.
395. Kuczenski R, Everall IP, Crews L, Adame A, Grant I, **Masliah E**. Escalating dose-multiple binge methamphetamine exposure results in degeneration of the neocortex and limbic system in the rat. Exp Neurol 207:42-51, 2007.
396. Wei J, Fujita M, Nakai M, Waragai M, Watabe K, Akatsu H, Rockenstein E, **Masliah E**, Hashimoto M. Enhanced lysosomal pathology caused by beta -synuclein mutants linked to dementia with lewy bodies. J Biol Chem 282:28904-14, 2007.
397. Nakashima-Yasuda H, Uryu K, Robinson J, Xie SX, Hurtig H, Duda JE, Arnold SE, Siderowf A, Grossman M, Leverenz JB, Woltjer R, Lopez OL, Hamilton R, Tsuang DW, Galasko D, **Masliah E**, Kaye J, Clark CM, Montine TJ, Lee VM, Trojanowski JQ. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol (Berl) 114:221-9, 2007
398. Waragai M, Nakai M, Wei J, Fujita M, Mizuno H, Ho G, **Masliah E**, Akatsu H, Yokochi F, Hashimoto M. Plasma levels of DJ-1 as a possible marker for progression of sporadic Parkinson's disease. Neurosci Lett 425:18-22, 2007.
399. Rockenstein E, Crews L, **Masliah E**. Transgenic animal models of neurodegenerative diseases and their application to treatment development. Adv Drug Deliv Rev 59:1093-102, 2007.
400. Fernagut PO, Hutson CB, Fleming SM, Tetreault NA, Salcedo J, **Masliah E**, Chesselet MF. Behavioral and histopathological consequences of paraquat intoxication in mice: Effects of alpha-synuclein over-expression. Synapse 61:991-1001, 2007.
401. Alirezaei M, Watry DD, Flynn CF, Kioussis WB, **Masliah E**, Williams BR, Kaul M, Lipton SA, Fox HS. Human immunodeficiency virus-1/surface glycoprotein 120 induces apoptosis through RNA-activated protein kinase signaling in neurons. J Neurosci 27:11047-55, 2007.
402. Spencer B, Rockenstein E, Crews L, Marr R, **Masliah E**. Novel strategies for Alzheimer's disease treatment. Expert Opin Biol Ther 7:1853-67, 2007.
403. Salaria S, Badkoobehi H, Rockenstein E, Crews L, Chana G, **Masliah E**, Everall IP, HNRC Group. Toll-like receptor pathway gene expression is associated with human immunodeficiency virus-associated neurodegeneration. J Neurovirol 13:496-503, 2007.
404. Kanazawa T, Chana G, Glatz SJ, Mizuno H, **Masliah E**, Yoneda H, Tsuang MT, Everall IP. The Utility of SELENBP1 gene expression as a biomarker for major psychotic disorders: Replication in schizophrenia and extension to bipolar disorder with psychosis. Am J Med Genet B Neuropsychiatr Genet 2007. [PMID: 18163446]
- 2008**
405. Kawahara K, Hashimoto M, Bar-On P, Ho GJ, Crews L, Mizuno H, Rockenstein E, Inam SZ, **Masliah E**. Alpha-Synuclein aggregates interfere with Parkin solubility and distribution: Role in pathogenesis of Parkinson's disease. J Biol Chem 283:6979-87, 2008. [PMID:18195004]
406. Sharikov Y, Walker RC, Greenberg J, Kouznetsova V, Nigam SK, Miller MA, **Masliah E**, Tsigelny IF. MAPAS: a tool for predicting membrane-contacting protein surfaces. Nat Methods 5:119, 2008. [PMID:18235431]

407. Winner B, Rockenstein E, Lie DC, Aigner R, Mante M, Bogdahn U, Couillard-Depres S, **Masliah E**, Winkler J. Mutant alpha-synuclein exacerbates age-related decrease of neurogenesis. Neurobiol Aging 29:913-25, 2008. [PMID: 17275140]
408. Belinson H, Lev D, **Masliah E**, Michaelson DM. Activation of the amyloid cascade in apolipoprotein E4 transgenic mice induces lysosomal activation and neurodegeneration resulting in marked cognitive deficits. J Neurosci 28:4690-701, 2008. [PMID: 18448646]
409. Crews L, Mizuno H, Desplats P, Rockenstein E, Adame A, Patrick C, Winner B, Winkler J, **Masliah E**. Alpha-synuclein alters Notch-1 expression and neurogenesis in mouse embryonic stem cells and in the hippocampus of transgenic mice. J Neurosci 16:4250-60, 2008. [PMID: 18417705]
410. Buxbaum JN, Ye Z, Reixach N, Friske L, Levy C, Das P, Golde T, **Masliah E**, Roberts AR, Bartfai T. Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of Abeta toxicity. Proc Natl Acad Sci USA 105:2681-6, 2008. [PMID: 18272491]
411. **Masliah E**. Neuropathology: Alzheimer's in real time. Nature 451:638-9, 2008. [PMID: 18256653]
412. Bar-On P, Crews L, Koob AO, Mizuno H, Adame A, Spencer B, **Masliah E**. Statins reduce neuronal alpha-synuclein aggregation in in vitro models of Parkinson's disease. J Neurochem 105:1656-67, 2008. [PMID:18248604]
413. Leverenz JB, Hamilton R, Tsuang DW, Schantz A, Vavrek D, Larson EB, Kukull WA, Lopez O, Galasko D, **Masliah E**, Kaye J, Woltjer R, Clark C, Trojanowski JQ, Montine TJ. Empiric Refinement of the pathologic assessment of lewy related pathology in the dementia patient. Brain Pathol 18:220-4, 2008. [PMID: 18241240]
414. Habib SL, Michel D, **Masliah E**, Thomas B, Ko HS, Dawson TM, Abboud H, Clark RA, Imam SZ. Role of tuberin in neuronal degeneration. Neurochem Res 33:1113-6, 2008. [PMID: 18320306]
415. Mocchetti I, Bachis A, **Masliah E**. Chemokine receptors and neurotrophic factors: potential therapy against aids dementia? J Neurosci Res 86:243-55, 2008. [PMID: 17847079]
416. Hult B, Chana G, **Masliah E**, Everall I. Neurobiology of HIV. Int Rev Psychiatry 20:3-13, 2008. [PMID: 18240058]
417. Pickford F, **Masliah E**, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, Wyss-Coray T. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. J Clin Invest 118:2190-99, 2008. [PMID: 18497889]
418. Doppler E, Rockenstein E, Ubhi K, Inglis C, Mante M, Adame A, Crews L, Hitzl M, Moesker H, **Masliah E**. Neurotrophic effects of Cerebrolysin in the Mecp2(308/Y) transgenic model of Rett syndrome. Acta Neuropathol. 2008. [PMID: 18600331]
419. Khanlou N, Moore DJ, Chana G, Cherner M, Lazzaretto D, Dawes S, Grant I, **Masliah E**, P Everall I. Increased frequency of alpha-synuclein in the substantia nigra in human immunodeficiency virus infection. J Neurovirol. 29:1-8, 2008.
420. Tsigelny IF, Sharikov Y, Miller MA, **Masliah E**. Mechanism of alpha synuclein oligomerization and membrane interaction: theoretical approach to unstructured proteins studies. Nanomedicine. 4:350-7, 2008. [PMID:1864077]
421. Jolivald CG, Lee CA, Beiswenger KK, Smith JL, Orlov M, Torrance MA, **Masliah E**. Defective insulin



- signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. J Neurosci Res. 15:3265-74, 2008 [
  422. Spencer B, Marr RA, Rockenstein E, Crews L, Adame A, Potkar R, Patrick C, Gage FH, Verma IM, **Masliah E**. Long-term neprilysin gene transfer is associated with reduced levels of intracellular Abeta and behavioral improvement in APP transgenic mice. BMC Neurosci. 9:109, 2008. [
  423. Hamilton JM, Salmon DP, Galasko D, Raman R, Emond J, Hansen LA, **Masliah E**, Thal LJ. Visuospatial deficits predict rate of cognitive decline in autopsy-verified dementia with Lewy bodies. Neuropsychology. 6:729-37, 2008.
  424. Kanazawa T, Chana G, Glatt SJ, Mizuno H, **Masliah E**, Yoneda H, Tsuang MT, Everall IP. The utility of SELENBP1 gene expression as a biomarker for major psychotic disorders: replication in schizophrenia and extension to bipolar disorder with psychosis. Am J Med Genet B Neuropsychiatr Genet. 6:686-9, 2008. [
  425. Tsigelny IF, Crews L, Desplats P, Shaked GM, Sharikov Y, Mizuno H, Spencer B, Rockenstein E, Trejo M, Platoshyn O, Yuan JX, **Masliah E**. Mechanisms of hybrid oligomer formation on the pathogenesis of combined Alzheimer's and Parkinson's Disease. PLoS ONE. 9:e3135, 2008. [PMID: 18769546]
  426. Ubhi K, Rockenstein E, Mante M, Patrick C, Adame A, Thukral M, Shults C, **Masliah E**. Rifampicin reduces alpha-synuclein in a transgenic mouse model of multiple systems atrophy. Neuroreport. 13:1271-6, 2008.
  427. Crews L, Lentz MR, Gonzalez RG, Fox HS, **Masliah E**. Neuronal injury in simian immunodeficiency virus and other animal models of neuroAIDS. J Neurovirol. 4:327-39, 2008. Review
  428. Fleming SM, Tetreault NA, Mulligan CK, Hutson CB, **Masliah E**, Chesselet MF. Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. Eur J Neurosci. 2:247-56, 2008.
  429. Crews L, Rockenstein E, **Masliah E**. Biological transgenic mouse models of Alzheimer's disease. Handb Clin Neurol. 89:291-301, 2008.
  430. Norman JP, Perry SW, Reynolds HM, Kieba M, De Mesy Bentley KL, Trejo M, Volsky DJ, Maggirwar SB, Dewhurst S, **Masliah E**, Gelbard HA. HIV-1 Tat activates neuronal ryanodine receptors with rapid induction of the unfolded protein response and mitochondrial hyperpolarization. PLoS ONE. 11:e3731, 2008.
- 2009**
431. Merrill DA, **Masliah E**, Roberts JA, McKay H, Kordower JH, Mufson EJ, Tuszyński MH. Association of early experience with neurodegeneration in aged primates. Neurobiol Aging.
  432. Achim CL, Adame A, Dumaop W, Everall IP, **Masliah E**; HNRC. Increased Accumulation of Intraneuronal Amyloid beta in HIV-infected Patients. J Neuroimmune Pharmacol. 2009.

433. Marongiu R, Spencer B, Crews L, Adame A, Patrick C, Trejo M, Dallapiccola B, Valente EM, **Masliah E**. Mutant Pink1 induces mitochondrial dysfunction in a neuronal cell model of Parkinson's disease by disturbing calcium flux. J Neurochem. 6:1561-74, 2009. [
434. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, **Masliah E**, Chiba AA, Tuszynski MH. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med. 3:331-7, 2009
435. Ubhi K, Rockenstein E, Doppler E, Mante M, Adame A, Patrick C, Trejo M, Crews L, Paulino A, Moessler H, **Masliah E**. Neurofibrillary and neurodegeneration pathology in APP-transgenic mice injected with AAV2-mutant TAU: neuroprotective effects of Cerebrolysin. Acta Neuropathol. 2009.
436. Belichenko PV, Wright EE, Belichenko NP, **Masliah E**, Li HH, Mobley WC, Francke U. Widespread changes in dendritic and axonal morphology in Mecp2-mutant mouse models of rett syndrome: Evidence for disruption of neuronal networks. J Comp Neurol. 3:240-258, 2009
437. Chu J, Hong NA, Masuda CA, Jenkins BV, Nelms KA, Goodnow CC, Glynne RJ, Wu H, **Masliah E**, Joazeiro CA, Kay SA. A mouse forward genetics screen identifies LISTERIN as an E3 ubiquitin ligase involved in neurodegeneration. Proc Natl Acad Sci U S A. 7:2097-103, 2009
438. Tatò ET, Everall IP, **Masliah E**, Hult BJ, Lucero G, Chana G, Soontornniyomkij V, Achim CL; HNRC. Differential Expression of Immunophilins FKBP51 and FKBP52 in the Frontal Cortex of HIV-Infected Patients with Major Depressive Disorder. J Neuroimmune Pharmacol. 2009
439. Belichenko PV, Kleschevnikov AM, **Masliah E**, Wu C, Takimoto-Kimura R, Salehi A, Mobley WC. Excitatory-inhibitory relationship in the fascia dentate in the Ts65Dn mouse model of Down syndrome. J Comp Neurol. 4:453-66, 2009.
440. Rose JB, Crews L, Rockenstein E, Adame A, Mante M, Hersh LB, Gage FH, Spencer B, Potkar R, Marr RA, **Masliah E**. Neuropeptide Y fragments derived from neprilysin processing are neuroprotective in a transgenic model of Alzheimer's disease. J Neurosci. 4:1115-25, 2009
441. Lamers SL, Salemi M, Galligan DC, de Oliveira T, Fogel GB, Granier SC, Zhao L, Brown JN, Morris A, **Masliah E**, McGrath MS. Extensive HIV-1 Intra-Host Recombination Is Common in tissues with abnormal histopathology. PLoS One. 4(3), 2009. [PMID: 19333384]
442. Wei J, Fujita M, Nakai M, Waragai M, Sekigawa A, Sugama S, Takenouchi T, **Masliah E**, Hashimoto M. Protective role of endogenous gangliosides for lysosomal pathology in a cellular model of synucleinopathies. Am J Pathol. 174(5):1891-909, 2009. [PMID: 19349362]
443. Watson JB, Hatami A, David H, **Masliah E**, Roberts K, Evans CE, Levine MS. Alterations in corticostriatal synaptic plasticity in mice overexpressing human alpha-synuclein. Neuroscience.

- 159(2):501-13, 2009. [PMID: 19361478]
444. Shaked GM, Chauv S, Ubhi K, Hansen LA, **Masliah E**. Interactions between the amyloid precursor protein C-terminal domain and G proteins mediate calcium dysregulation and amyloid beta toxicity in Alzheimer's disease. FEBS J. 276(10):2736-51, 2009. [PMID: 19368557]
445. Crews L, Patrick C, Achim CL, Everall IP, **Masliah E**. Molecular pathology of Neuro-AIDS (CNS-HIV). Int J Mol Sci. 10(3):1045-63, 2009. [PMID: 19399237]
446. Ubhi K, Lee PH, Adame A, Inglis C, Mante M, Rockenstein E, Stefanova N, Wenning GK, **Masliah E**. Mitochondrial inhibitor 3-nitropropionic acid enhances oxidative modification of alpha-synuclein in a transgenic mouse model of multiple systems atrophy. J Neurosci Res. 87(12):2728-39, 2009. [PMID: 19405128]
447. Bengoechea TG, Chen Z, O'Leary D, **Masliah E**, Lee KF. P75 reduces beta-amyloid-induced sympathetic innervation deficits in an Alzheimer's disease mouse model. Proc Natl Acad Sci U S A. 106(19):7870-5, 2009. [PMID: 19416837]
448. Cartier AE, Djakovic SN, Salehi A, Wilson SM, **Masliah E**, Patrick GN. Regulation of synaptic structure by ubiquitin C-terminal hydrolase L1. J Neurosci. 29(24):7857-68, 2009. [PMID: 19535597]
449. Crews L, Tsigelny I, Hashimoto M, **Masliah E**. Role of synucleins in alzheimer's disease. Neurotox Res. (3):306-17, 2009. [PMID: 19551456]
450. Dziejczapolski G, Glogowski CM, **Masliah E**, Heinemann SF. Deletion of the alpha 7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease. J Neurosci. 29(27):8805-15, 2009. [PMID: 19587288]
451. Wacker JL, Huang SY, Steele AD, Aron R, Lotz GP, Nguyen Q, Giorgini F, Roberson ED, Lindquist S, **Masliah E**, Muchowski PJ. Loss of Hsp70 exacerbates pathogenesis but not levels of fibrillar aggregates in a mouse model of Huntington's disease. J Neurosci. 29(28):9104-14, 2009. [PMID: 19605647]
452. Winner B, Desplats P, Hagl C, Klucken J, Aigner R, Ploetz S, Laemke J, Karl A, Aigner L, **Masliah E**, Buerger E, Winkler J. Dopamine receptor activation promotes adult neurogenesis in an acute Parkinson model. Exp Neurol. 219(2):543-52, 2009. [PMID: 19619535]
453. Baughn MR, Vaux K, **Masliah E**. Placenta Accreta in a Separate Uterine Horn. Pediatr Dev Pathol. 28:1, 2009. [PMID: 19642813]
454. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, **Masliah E**, Lee SJ. Inclusion formation and neuronal cell death through neuron-to neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A. 106(31):13010-5, 2009. [PMID: 19651612]
455. Shankar GM, Leissring MA, Adame A, Sun X, Spooner E, **Masliah E**, Selkoe DJ, Lemere CA, Walsh DM. Biochemical and immunohistochemical analysis of an Alzheimer's disease mouse model reveals the presence of multiple cerebral Abeta assembly forms throughout life. Neurobiol Dis. 36(2):293-302, 2009. [PMID: 19660551]

456. Nguyen TB, Lucero GR, Chana G, Hult BJ, Tatro ET, **Masliah E**, Grant I, Achim CL, Everall IP. Glycogen synthase kinase-3beta (GSK-3beta) inhibitors AR-A014418 and B6B30 prevent human immunodeficiency virus-mediated neurotoxicity in primary human neurons. *J Neurovirol.* 17:1-5, 2009. [PMID: 19688630]
457. Everall I, Vaida F, Khanlou N, Lazzaretto D, Achim C, Letendre S, Moore D, Ellis R, Cherne M, Gelman B, Morgello S, Singer E, Grant I, **Masliah E**. Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. *J Neurovirol.* 8:1-11, 2009. [PMID: 19739020]
458. Spencer B, Potkar R, Trejo M, Rockenstein E, Patrick C, Gindi R, Adame A, Wyss-Coray T, **Masliah E**. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body disease. *J Neurosci.* 28;29(43):13578-88, 2009. [PMID: 19864570]
459. Mbefo MK, Paleologou KE, Boucharaba A, Oueslati A, Schell H, Fournier M, Olschewski D, Yin G, Zweckstetter M, **Masliah E**, Kahle PJ, Hirling H, Lashuel HA. Phosphorylation of synucleins by members of the Polo-like kinase family. *J Biol Chem.* 22;285(4):2807-22, 2009. [PMID: 19889641]
460. Jolivald CG, Hurford R, Lee CA, Dumaop W, Rockenstein E, **Masliah E**. Type 1 diabetes exaggerates features of Alzheimer's disease in APP transgenic mice. *Exp Neurol.* 2009. [PMID: 19931251]
461. Koob AO, Paulino AD, **Masliah E**. GFAP reactivity, apolipoprotein E redistribution and cholesterol reduction in human astrocytes treated with alpha-synuclein. *Neurosci Lett.* 18;469(1):11-4. 2009. [PMID: 19932737]
462. Koob AO, Ubhi K, Paulsson JF, Kelly J, Rockenstein E, Mante M, Adame A, **Masliah E**. Lovastatin ameliorates alpha-synuclein accumulation and oxidation in transgenic mouse models of alpha-synucleinopathies. *Exp Neurol.* 221(2):267-74. 2009. [PMID: 19944097]
463. Crews L, Rockenstein E, **Masliah E**. APP transgenic modeling of Alzheimer's disease: mechanism of neurodegeneration and aberrant neurogenesis. *Brain Struct Funct.* 2009. [PMID: 20091183]
464. Cohen E, Paulsson JF, Blinder P, Burstyn-Cohen T, Du D, Estepa G, Adame A, Pham HM, Holzenberger M, Kelly JW, **Masliah E**, Dillin A. Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell.* 139(6):1157-69. 2009. [PMID: 20005808]
465. Wu N, Joshi PR, Cepeda C, **Masliah E**, Levine MS. Alpha-synuclein overexpression in mice alters synaptic communication in the corticostriatal pathway. *J Neurosci Res.* 2009. [PMID: 20029978]

## 2010

466. Harris JA, Devidze N, Halabisky B, Lo I, Thwin MT, Yu GQ, Breiden DE, **Masliah E**, Mucke L. Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein. *J Neurosci.* 30(1):372-81. 2010. [PMID: 20053918]
467. Lee HJ, Suk JE, Patrick C, Bae EJ, Cho JH, Rho S, Hwang D, **Masliah E**, Lee SJ. Direct transfer of alpha-

synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem. 2010. [PMID: 20071342]

468. Lemere CA, **Masliah E**. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? Nat Rev Neurol. 6(2):108-19. 2010. [PMID: 20140000]

#### **Submitted:**

441. Pham T, Benirschke B, Jones K, Mannino F, **Masliah E**. Heterogeneity of neuromuscular pathology in the Pena-Shokier phenotype. Pediatr Dev Path 2006.
442. Overstreet K, Weidner N, Herndier B, and **Masliah E**. CD20 negative, small lymphocytic lymphoma (SLL) following anti-CD20 monoclonal antibody therapy: an autopsy study. Modern Pathology 2007.
443. Bar-On P, Rockenstein E, Hashimoto M, **Masliah E**. Advances in gene transfer and pharmacological regulation of protein aggregation in the treatment of Alzheimer's and Parkinson's diseases. J Alzheimer Dis 2007.
444. Marr AR, Rockenstein E, Kindy MS, Hersh LB, Gage FH, Verma IM, **Masliah E**. Long-term expression of Neprilysin reduces amyloid deposition and the associated pathology in APP-transgenic mice. 2006.
445. Price DL, Rockenstein E, Phung V, MacLean NA, Askay D, Paulino A, **Masliah E**, Ellisman MH. Increased mGluR5 receptor immunoreactivity in CNS of Parkinsonian patients and transgenic mice overexpressing alpha-synuclein. 2007.

#### **Letters to the Editor:**

1. Hansen L, Galasko D, Katzmen R, Thal L, Alford M, Salmon D, **Masliah E**, DeTeresa R, Butters N. Reply from the Authors (Lewy body Variant). Neurology 40: 1148-9, 1990.
2. **Masliah E**, Hansen L. Reply (Atypical PSP). Ann Neurol. 30:854, 1991.
3. **Masliah E**, Saitoh T. Lewy bodies, Alzheimer pathology, and APP mutation. Neurosci Lett 180:292-3, 1993.
4. Lipton SA, Brenneman DE, Silverstein FS, **Masliah E**, Mucke L. gp120 and neurotoxicity *in vivo*. Trends Pharmacol Sci 16:122, 1995.
5. McKeel DW Jr, **Masliah E**, Hansen LA, and Morris JC. Commentary on "Synaptic Pathology in Prefrontal Cortex Is Present only with Severe Dementia in Alzheimer Disease" (JNEN 2001; 60; 929-36). J Neuropathol Exp Neurol. 61:295-6, 2002.

#### **Book chapters:**

1. Terry RD, **Masliah E**. The promise of confocal laser scanning microscopy. In: Neuropathology in Brain Research (Ikuta F, Ed) Elsevier Science Publishers Amsterdam. p 15, 1991.
2. Hessler D, Young SJ, Carragher BO, Martone M, Hinshaw JE, Milligan RA, **Masliah E**, Whittaker M, Lamont S, Ellisman M. SYNU: Software for visualization of 3-dimensional biological structures. Microscopy: The Key Research Tool 22:73-82, 1992.

3. Saitoh T, **Masliah E**. Protein kinase alterations are early pathological markers of Alzheimer's disease. In: *Alzheimer's Disease: Advances in Clinical and Basic Research* (Corain B, Ed) John Wiley and Sons, Ltd., New York. p 349, 1993.
4. **Masliah E**, Achim C, De Teresa R, Ge N, Wiley C. Cellular neuropathology in HIV encephalitis. In: *HIV, AIDS and the Brain* (Price RW, Ed) Raven Press, New York. p 119, 1994.
5. Terry RD, Hansen L, **Masliah E**. Structural alterations in Alzheimer Disease. In: *Alzheimer Disease* (Terry RD, Katzman R, Eds.) Raven Press, New York. p 179, 1994.
6. Saitoh T, Roch J-M, Jin L-W, Ninomiya H, Otero DAC, Yamamoto K, **Masliah E**. The biological function of amyloid  $\beta$ /A4 protein precursor. In: *Amyloid protein precursor in development, aging and Alzheimer's disease*. (Masters CL, Beyreuther K, Trillet M, Christen Y, Eds) Fondation IPSEN. Springer-Verlag, New York. p 90, 1994.
7. **Masliah E**, Saitoh T. Protein kinase C in Alzheimer's disease. In: *Protein kinase C in the CNS, focus on neuronal plasticity. Proceedings.* (Canonica PL, Scapagnini U, Pamparana F, Routtenberg A, Eds) Masson, Milano. p 101, 1994.
8. **Masliah E**, Ueda K, Mucke L. Pathophysiological Basis of Alzheimer's disease. In: *Proceedings of the XVII World Congress of Anatomic and Clinical Pathology*. Monduzzi Editore, Bologna. p 315, 1994.
9. Terry RD, **Masliah E**. Synaptic pathology in the pathogenesis of Alzheimer dementia. In: *New Trends in the diagnosis and therapy of Alzheimer's disease*. (Jellinger KA, Ladurner G, Windisch M, Eds) Springer-Verlag, Austria. p 1, 1994.
10. Mucke L, **Masliah E**, Campbell IL. Transgenic models to assess the neuropathogenic potential of HIV-1 proteins and cytokines. In: *HIV and dementia* (Oldstone MBA, Vitkovic L, Eds) Springer-Verlag, Berlin. p 187, 1995.
11. **Masliah E**, Mallory M, Alford M, Ge N, Mucke L. Abnormal synaptic regeneration in hAPP695 transgenic and APOE knockout mice. In: *Research Advances in Alzheimer's Disease and Related Disorders* (Iqbal K, Mortimer J, Winblad B, Wisniewski H, Eds). p 405, 1995.
12. **Masliah E**, Mallory M, Alford M, Veinbergs I, Roses AD. ApoE role in maintaining the integrity of the aging central nervous system. In: *Apolipoprotein E and Alzheimer's disease*. (Christen Y, Roses AD, Eds) Springer-Verlag, New York. p 59, 1996.
13. Gold LH, Heyser CJ, Roberts A, Henriksen SJ, Steffensen SC, Siggins GR, Bellinger FP, Chiang C-S, Powell HC, **Masliah E**, Campbell IL. Behavioral and neurophysiological effects of CNS expression of cytokines in transgenic mice. In: *Proceedings of the symposium on AIDS, Drugs of Abuse and Neuroimmune axis*. (Friedmann H, Ed) Plenum Press, New York. 1996.
14. Everall IP, Gray F, **Masliah E**. Neuronal injury and apoptosis. In: *The Neurology of AIDS*. (Gendelman HE, Lipton SA, Epstein L, Swindells S, Eds) Chapman & Hall, New York. p 261, 1996.
15. Games D, **Masliah E**, Lee M, Johnson-Wood K, Schenk D. Neurodegenerative Alzheimer-like pathology in PDAPP 717V---->F transgenic mice. In: *Connections, cognition and Alzheimer's disease*. (Hyman B, Duyckaerts C, Christen Y, Eds) Springer-Verlag, New York. p 105, 1997.
16. **Masliah E**, Mallory M, Alford M, DeTeresa R, Iwai A, Saitoh T. Molecular mechanisms of synaptic disconnection in Alzheimer Disease. In: *Connections, cognition and Alzheimer's disease*. (Hyman B, Duyckaerts C, Christen Y, Eds) Springer-Verlag, New York. p 121, 1997.

17. **Masliah E**, Alford M. Assays for the analysis of synaptic proteins in neurodegenerative disorders. In: Methods in Molecular Medicine, vol. 22. (Harry J, Tilson HA, Eds) Humana Press Inc., Totowa, NJ. p 201, 1998.
18. Pagenstecher A, **Masliah E**, Stalder AK, Campbell IL. Transgenic mice expressing cytokines in the CNS as model systems for the study of inflammatory neurodegenerative and demyelinating disorders. Chapter 6. In: Neuroimmunodegeneration. (Wong PKY, Lynn WS, Eds) Springer-Verlag and RG Landes Company, New York. p 115, 1998.
19. **Masliah E**, Salmon DP. Neuropathological correlates of dementia in Alzheimer's disease. In: Cerebral Cortex, vol. 14. p 513, 1999.
20. **Masliah E**, Licastro F. Neuronal and synaptic loss, reactive gliosis, microglial response and induction of the complement cascade. In: Neurodegenerative dementias. (Clark L, Trojanowski JQ, Eds) McGraw-Hill, New York. 1999.
21. **Masliah E**. Transgenic animal models of Alzheimer's disease. In: Alzheimer's disease. Second edition. (Terry RD, Sisodia S, Eds) Raven Press, New York. p 245, 1999.
22. Wyss-Coray T, Lin C, Von Euw D, **Masliah E**, Mucke L, Lacombe P. Alzheimer's disease-like cerebrovascular pathology in transforming growth factor- $\beta$ 1 transgenic mice and functional metabolic correlates. In: Vascular factors in Alzheimer's disease vol 903. p 317, 2000.
23. Hansen L, **Masliah E**. Neurobiology of disorders with Lewy bodies. In: Functional Neurobiology of Aging (Hof PR, Mobbs CV, Eds) Academic Press, New York. p 248, 2001.
24. Hashimoto M, Rockenstein E, Takenouchi T, Mallory M, **Masliah E**. Mechanisms of  $\alpha$ -synuclein and NAC fibrillogenesis. In: Alzheimer's disease: Advances in etiology, pathogenesis and therapeutics. (Iqbal K, Sisodia SS, Winblad B, Eds) John Wiley & Sons, Ltd., England, pp. 569-86, 2002.
25. **Masliah E**. Degenerative neural diseases, animal models. In: Yearbook of Science & Technology. McGraw-Hill, pp. 59-61, 2002.
26. Langford TD, Everall IP, **Masliah E**. Current concepts in HIV neuropathogenesis: Neuronal injury, white matter disease, and neurotrophic factors. In: The Neurology of AIDS, 2002.
27. Ellis RJ, Merdes A, **Masliah E**, Langford TD. Clinical-neuropathologic correlation in HIV in the era of highly active antiretroviral therapy. In: The Neurology of AIDS, Second edition. (Gendelman HE, Grant I, Everall IP, Lipton S, Swindells S.). Oxford University Press, United Kingdom. pp. 801-809, 2005.
28. Langford TD, Everall IP, **Masliah E**. Current concepts in HIV neuropathogenesis: neuronal injury, white matter disease, and neurotrophic factors. In: The Neurology of AIDS, Second edition. (Gendelman HE, Grant I, Everall IP, Lipton S, Swindells S.). Oxford University Press, United Kingdom. pp. 405-414, 2005.
29. Kandaneeratchi A, Nath A, Lipton S, **Masliah E**, Everall IP. Protection against HIV-1 gp120 and HIV-1 Tat neurotoxicity. In: The Neurology of AIDS, Second edition. (Gendelman HE, Grant I, Everall IP, Lipton S, Swindells S.). Oxford University Press, United Kingdom. pp.200-210, 2005
30. **Masliah E**. Anatomical Network: Structure and Function of the Nervous System. In Neuroimmune Pharmacology (Edited by Ikezu T and Gendelman HE) Springer 2008
31. Brundin P., Winkler J., and **Masliah E**. Adult Neurogenesis in Neurodegenerative Diseases. In Adult Neurogenesis (Edited by Gage FH, Kempermann G, and Song H) Cold Spring Harbor Laboratory Press 2008.

32. Crews L, Rockenstein E, and **Masliah E.**, Biological Transgenic Mouse Models of Alzheimer's Disease. In Dementias (Edited by Duyckaerts C and Litvan C). In Handbook of Clinical Neurology (Edited by Aminoff MJ, Boller F, and Swaab DF) (Volume 89) Elsevier 2008.
33. **Masliah E.**, Crews L. Genetically Engineered Mouse Models of Neurodegenerative Disorders. In Protein Misfolding, Aggregation, and Conformational Diseases. Part A: Protein Aggregation and Conformational Diseases (Edited by Uversky VN and Fink AL) Springer 2006.
34. Crews L, Spencer B, **Masliah E.**, Role of Abeta Degrading Enzymes in Synaptic Plasticity and Neurogenesis in Alzheimer's Disease. In Current Hypotheses and Research Milestones in Alzheimer's Disease(Edited by Maccioni RB and Perry G) Springer 2009.

#### **Patents:**

1. **Masliah E**, Katzman R. "*In vivo* imaging of amyloid and synapses in the brain with radio probes". UC Case #93-298-1.
2. **Masliah E.** "Novel component of amyloid in Alzheimer's disease and methods for use of same". PCT #WO 95/06407, UC Case #1991-082.
3. Mucke L, Wyss-Coray T, **Masliah E.** "Transgenic animal models of Alzheimer's disease and cerebral amyloid angiopathy". US Patent #6,175,057B1, UC Case #1997-215.
4. **Masliah E.** "Methods for screening for anti-amyloidogenic properties and method for treatment of neurodegenerative diseases". PCT #WO 00/20020, UC Cases #1999-013 & #1999-014.
5. **Masliah E.** "RiboProbe". UC Case #SD2000-078.
6. **Masliah E.** "Transgenic animal model of Parkinson's Disease". UC Case #SD2000-141.
7. **Masliah E**, Rockenstein E, Mallory M. "Transgenic mice expressing human APP and human  $\alpha$ -synuclein". Patent #5,612,486, UC Case #SD2001-071.
8. **Masliah E**, Rockenstein E. "Flourescent-tagged synaptic proteins as models for neurodegenerative disorders". UC Case #SD2001-019.
9. **Masliah E**, Shultz C. "Diagnostic Test for Parkinson's disease". UC Case SD# 2001-191.
10. **Masliah E.** "Development of an Anti-Parkinson's Vaccine in Transgenic Mice". UC Case #SD2002-035.
11. **Masliah E.** "Treatment of Neurodegenerative Diseases and Dementing Disorders by Inhibiting Protein Aggregation: Use of W-Guanidino Compounds". UC Case #SD2002-063.
12. **Masliah E.** "Treatment of Neurodegenerative Diseases and Dementing Disorders by Inhibiting Glycogen synthase kinase 3-Beta (GSK3 $\beta$ ): Use of Lithium Chloride to Inhibit HIV pg120 Neurotoxicity". UC Case #SD2002-064.
13. **Masliah E.** "Treatment of Neurodegenerative Diseases and Dementing Disorders by Inhibiting Protein Aggregation: Use of Gene Therapy with the Beta Synuclein Gene for Treating Parkinson's Disease". UC Case #SD2002-065.



## Research

## Open Access

## Passive immunotherapy against A $\beta$ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage

Donna M Wilcock<sup>1</sup>, Aryn Rojiani<sup>2</sup>, Arnon Rosenthal<sup>3</sup>, Sangeetha Subbarao<sup>3</sup>, Melissa J Freeman<sup>1</sup>, Marcia N Gordon<sup>1</sup> and Dave Morgan<sup>\*1</sup>

Address: <sup>1</sup>Alzheimer's Research Laboratory, University of South Florida, Department of Pharmacology, 12901 Bruce B Downs Blvd, Tampa, Florida 33612, USA, <sup>2</sup>Alzheimer's Research Laboratory, University of South Florida, Department of Interdisciplinary Oncology, 12901 Bruce B Downs Blvd, Tampa, Florida 33612, USA and <sup>3</sup>Rinat Neuroscience Corp., 3155 Porter Drive, Palo Alto, California 94304, USA

Email: Donna M Wilcock - dwilcock@hsc.usf.edu; Aryn Rojiani - arojiani@hsc.usf.edu; Arnon Rosenthal - ar@rinatneuro.com; Sangeetha Subbarao - Sangeetha@rinatneuro.com; Melissa J Freeman - mlfreema1@hsc.usf.edu; Marcia N Gordon - mgordon@hsc.usf.edu; Dave Morgan - dmorgan@hsc.usf.edu

\* Corresponding author

Published: 08 December 2004

Received: 10 November 2004

Accepted: 08 December 2004

Journal of Neuroinflammation 2004, 1:24 doi:10.1186/1742-2094-1-24

This article is available from: <http://www.jneuroinflammation.com/content/1/1/24>

© 2004 Wilcock et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

**Background:** Anti-A $\beta$  immunotherapy in transgenic mice reduces both diffuse and compact amyloid deposits, improves memory function and clears early-stage phospho-tau aggregates. As most Alzheimer disease cases occur well past midlife, the current study examined adoptive transfer of anti-A $\beta$  antibodies to 19- and 23-month old APP-transgenic mice.

**Methods:** We investigated the effects of weekly anti-A $\beta$  antibody treatment on radial-arm water-maze performance, parenchymal and vascular amyloid loads, and the presence of microhemorrhage in the brain. 19-month-old mice were treated for 1, 2 or 3 months while 23-month-old mice were treated for 5 months. Only the 23-month-old mice were subject to radial-arm water-maze testing.

**Results:** After 3 months of weekly injections, this passive immunization protocol completely reversed learning and memory deficits in these mice, a benefit that was undiminished after 5 months of treatment. Dramatic reductions of diffuse A $\beta$  immunostaining and parenchymal Congophilic amyloid deposits were observed after five months, indicating that even well-established amyloid deposits are susceptible to immunotherapy. However, cerebral amyloid angiopathy increased substantially with immunotherapy, and some deposits were associated with microhemorrhage. Reanalysis of results collected from an earlier time-course study demonstrated that these increases in vascular deposits were dependent on the duration of immunotherapy.

**Conclusions:** The cognitive benefits of passive immunotherapy persist in spite of the presence of vascular amyloid and small hemorrhages. These data suggest that clinical trials evaluating such treatments will require precautions to minimize potential adverse events associated with microhemorrhage.

## Background

Alzheimer's disease is characterized not only by the presence of parenchymal amyloid deposits and intracellular tangles but also by the presence of amyloid deposits in the vasculature, a condition referred to as cerebral amyloid angiopathy (CAA). The CAA observed in both Alzheimer's disease patients [1] and some of the transgenic mouse models [2] is primarily composed of the shorter form of amyloid beta ( $A\beta$ ),  $A\beta_{1-40}$ , while the majority of amyloid deposits in the parenchyma are composed of  $A\beta_{1-42}$ , although the compact amyloid deposits also contain  $A\beta_{1-40}$ .

40\*

Anti- $A\beta$  immunotherapy has been considered as a potential treatment for Alzheimer's disease for some time [3,4]. Active immunization with a vaccine including  $A\beta_{1-42}$  fibrils progressed to human clinical trials where its administration was suspended due to meningoencephalitis in a subset of patients [5]. To date there have been pathology reports on two patients who participated in the trial and subsequently died [6,7]. Both reports note that while the numbers of parenchymal amyloid deposits appeared lower than expected in these cases, the CAA in these patients did not appear outside the normal range for Alzheimer's disease. In addition, one report mentioned multiple cortical hemorrhages and the presence of hemosiderin around the CAA vessels [7].

Given the adverse reactions to the active immunization, the irreversibility of such procedures and the variable antibody response to vaccines in older individuals [8], passive immunization against the  $A\beta$  peptide emerged as an alternative immunotherapeutic strategy. Studies in young and middle aged APP-transgenic mice have reported significant amyloid reductions with passive immunization [9-11]. Such treatments also demonstrate rapid improvements of memory function in APP-transgenic mice, sometimes without detectable reductions in amyloid [12-14]. Most recently, intracranial administration of anti- $A\beta$  antibodies has been shown to not only remove  $A\beta$  but also clear, early-stage, hyperphosphorylated-tau aggregates [15]. Importantly, in the only prior study evaluating adoptive antibody transfer in older APP-transgenic mice, Pfeifer et al. [16] reported a doubling of cerebral hemorrhages associated with significant reductions in amyloid burden after administration of an N-terminal specific anti- $A\beta$  antibody.

## Materials and Methods

### Experiment design

Mice derived from APP Tg2576 mice were obtained from our breeding program at University of South Florida started in 1996 [17]. For the 5-month treatment study, 13 APP-transgenic mice, aged 23 months, were assigned to one of two groups. The first group received weekly intra-

peritoneal anti- $A\beta$  antibody injections (antibody 2286; mouse-monoclonal anti-human  $A\beta_{25-40}$  IgG1; Rinat Neurosciences, Palo Alto, CA) for a period of 5 months ( $n = 6$ ). The second group received weekly intraperitoneal anti-AMN antibody (2906; mouse-monoclonal anti-*Drosophila* amnesiac protein IgG1; Rinat Neurosciences, Palo Alto, CA) injections for a period of 5 months ( $n = 7$ ). Seven nontransgenic mice were also assigned to one of two groups. The first group received weekly intraperitoneal anti- $A\beta$  antibody injections for a period of 5 months ( $n = 4$ ). The second group received weekly intraperitoneal anti-AMN antibody injections for a period of 5 months ( $n = 3$ ).

For the time course study of 1-, 2- or 3-month treatment, 22 APP-transgenic mice aged 19 months were assigned to one of four experimental groups, as described previously [14]. The first three groups received weekly intraperitoneal anti- $A\beta$  antibody injections for 3 months, 2 months or 1 month, ending when all mice were 22 months of age. The fourth group received weekly intraperitoneal anti-AMN antibody injections for 3 months.

### Behavioral analysis

Following 3 and 5 months of treatment, the mice from the 5-month study were subjected to a two-day radial-arm water-maze paradigm. The apparatus was a 6-arm maze as described previously [18]. On day one, 15 trials were run in three blocks of 5. A cohort of 4 mice were run sequentially for each block (i.e., each of 4 mice get trial one, then the same mice get trial two, etc.). After each 5-trial block, a second cohort of mice was run permitting an extended rest period before mice were exposed to the second block of 5 trials. The goal arm was different for each mouse in a cohort to minimize odor cues. The start arm was varied for each trial, with the goal arm remaining constant for a given individual for both days. For the first 11 trials, the platform was alternately visible then hidden (hidden for the last 4 trials). On day two, the mice were run in exactly the same manner as day one except that the platform was hidden for all trials. The number of errors (incorrect arm entries) was measured in a one-minute time frame. As standard practice, mice failing to make an arm choice in 20 seconds are assigned one error, but no mice in this study had to be assigned an error in this manner. The same individual administered the antibody treatments and placed mice in the radial-arm water maze. Due to the numbers of mice in the study the researcher was unaware of treatment group identity of each mouse. Also, the dependent measures in the radial-arm water-maze task are quantitative, not evaluative, so the potential for tester bias is reduced. In order to minimize the influence of individual trial variability, each mouse's errors for 3 consecutive trials were averaged producing 5 data points for each day, which were analyzed statistically by ANOVA using StatView (SAS Institute Inc., NC).

### Tissue preparation and histology

On the day of sacrifice mice were weighed, overdosed with 100 mg/kg Nembutal (Abbott laboratories, North Chicago, IL), and then intracardially perfused with 25 mL of 0.9% sodium chloride. Brains were rapidly removed, and the left half of the brain was immersion fixed for 24 h in freshly prepared 4% paraformaldehyde in 100 mM KPO<sub>4</sub> (pH 7.2) for histopathology. The hemi-brains were then incubated for 24 h in 10%, 20% and 30% sucrose sequentially for cyroprotection. Horizontal sections of 25  $\mu$  thickness were collected using a sliding microtome and stored at 4°C in Dulbecco's phosphate-buffered saline with sodium azide (pH 7.2) to prevent microbial growth. A series of 8 equally spaced tissue sections 600  $\mu$  apart were randomly selected spanning the entire brain and stained using free-floating immunohistochemistry for total A $\beta$  (rabbit polyclonal anti-pan A $\beta$ ; Biosource, Camarillo, CA, 1:10,000) as previously described [2,14]. A second series of tissue sections 600  $\mu$  apart were stained using 0.2% Congo red in NaCl-saturated 80% ethanol. Another set of sections were also mounted and stained for hemosiderin using 2% potassium ferrocyanide in 2% hydrochloric acid for 15 min, followed by a counterstain in a 1% neutral red solution for 10 min. Quantification of Congo red staining and A $\beta$  immunohistochemistry was performed using the Image-Pro Plus (Media Cybernetics, Silver Spring, MD) to analyze the percent area occupied by positive stain. One region of the frontal cortex and three regions of the hippocampus were analyzed (to ensure that there was no regional bias in the hippocampal values). The initial analysis of Congo red was performed to give a total value. A second analysis was performed after manually editing out all of the parenchymal amyloid deposits to yield a percent area restricted to vascular Congo red staining. To estimate the parenchymal area of Congo red, we subtracted the vascular amyloid values from the total percentage. For the hemosiderin stain the numbers of Prussian blue-positive sites were counted on all sections and the average number of sites per section calculated. Looking at the sections at a low magnification we were able to observe a qualitative differences between animals; however, the percent area was so low that many fields contained no positive stain. Eight equally spaced sections were examined and the number of positive profiles was determined and averaged to a per-section value. To assess possible treatment-related differences, the values for each treatment group were analyzed by one-way ANOVA followed by Fisher's LSD means comparisons.

## Results

### Reversal of cognitive deficits by passive amyloid immunotherapy

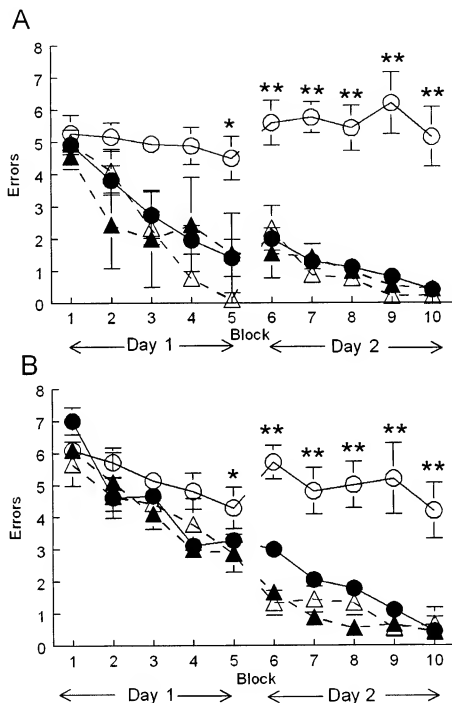
The radial-arm water-maze task detects spatial learning and memory deficits in transgenic mouse models [18,19].

We treated 23-month-old mice for 5 months with anti-A $\beta$  2286 or control antibody 2906 (against a *Drosophila*-specific protein) and tested them for spatial navigation learning in a two-day version of the radial-arm water maze after 3 months of treatment and, using a new platform location, again after 5 months of treatment. At both testing times we found that APP-transgenic mice treated with the control antibody failed to learn platform location over two days of testing and were significantly impaired compared to the nontransgenic mice treated with either antibody (Fig. 1). However, APP-transgenic mice administered the anti-A $\beta$  antibodies demonstrated a complete reversal of the impairment observed in the control-treated APP-transgenic mice, ending day two with a mean performance near 0.5 errors per trial (Fig. 1). Although learning at the later time point, when the mice were 28 months of age, may have been slightly slower for all groups, there was no impairment of the anti-A $\beta$  antibody-treated APP.

### Passive amyloid immunotherapy clears parenchymal A $\beta$ deposits, but increases vascular amyloid

In a prior experiment examining the effects of passive anti-A $\beta$  immunotherapy for 1, 2 or 3 months in APP-transgenic mice killed at 21 months of age [14], we found a time-dependent reduction of both A $\beta$  immunostaining of diffuse and fibrillar deposits and Congo-red staining of fibrillar amyloid deposits. In the current study we found a similar reduction in both A $\beta$  immunostaining (Table 1) and total Congo-red staining (Fig. 2A, left panel;  $p < 0.001$  frontal cortex and  $p < 0.01$  hippocampus) after 5 months of immunotherapy. We noted that the bulk of what remained was vascular amyloid. We then separately analyzed vascular and parenchymal deposits which revealed a near 90% reduction in parenchymal deposits ( $p < 0.001$ ) but a 3–4 fold elevation of vascular Congo-red staining ( $p < 0.0001$ ; Fig. 2A, center and right panels, respectively). We also separately analyzed vascular and parenchymal Congo-red staining on mice from our earlier study [14], treated passively for 1, 2 or 3 months with anti-A $\beta$  or control antibody, and found a similar result. There was a graded reduction in overall Congo-red staining nearing 75% as duration of antibody exposure increased (as reported previously; Fig. 2B). However, when separated into vascular Congo-red deposits and parenchymal deposits, there was an antibody-exposure-time-dependent increase in vascular deposition in both hippocampus and frontal cortex (Fig. 2C;  $p < 0.05$  frontal cortex and hippocampus) and a corresponding nearly 90% decrease in parenchymal deposits (Fig. 2D;  $p < 0.001$  in frontal cortex and hippocampus).

These differences were readily observed examining micrographs of sections from these mice. Mice treated with control antibodies revealed occasional cortical vascular

**Figure 1**

Spatial learning deficits in APP-transgenic mice were reversed following 3 and 5 months of immunization. Mice were tested in a two-day version of the radial-arm water maze. Solid lines represent APP-transgenic mice while dashed lines represent non-transgenic mice. Open symbols indicate anti-AMN, control-antibody treatment (○: APP-transgenic, control antibody; △: non-transgenic, control antibody) while closed symbols indicate anti-Aβ antibody treatment (●: APP-transgenic, Aβ antibody; ▲: non-transgenic, Aβ antibody). Panel A shows mean number of errors made over the two-day trial period following 3 months of immunization. Each data point is the average of 3 trials. Panel B shows the mean number of errors made over the 2-day trial period following 5 months of immunization. For both graphs \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.001$  when the APP-transgenic mice receiving control antibody are compared with the remaining groups.

**Table 1: Total A $\beta$  load is significantly reduced following 5 months of anti-A $\beta$  antibody treatment. Percent area occupied by positive immunohistochemical stain for A $\beta$  is shown  $\pm$  standard error of the mean for both the frontal cortex and hippocampus. Also shown is the percent reduction of A $\beta$  observed following anti-A $\beta$  antibody treatment**

Region	% area positive for A $\beta$ : control treated	% area positive for A $\beta$ : anti-A $\beta$ treated	% reduction following anti-A $\beta$ antibody treatment
Frontal Cortex	34.855 $\pm$ 2.265	9.681 $\pm$ 0.754	72
Hippocampus	23.994 $\pm$ 0.985	8.212 $\pm$ 0.596	66

amyloid deposits (22 months, Fig. 3A, 28 months, Fig. 3C), while mice administered anti-A $\beta$  antibodies had increased amounts of vascular amyloid staining (3-month treatment, Fig 3B; 5-month treatment, Fig 3D). Those vessels containing amyloid following treatment with anti-A $\beta$  antibody also exhibited apparent increases in microglial activation as measured by CD45 expression (Fig. 3F) compared to mice treated with control antibody (Fig. 3E). Unfortunately, the shifting numbers and sizes of vascular and parenchymal deposits caused by the antibody therapy greatly complicated measurement of microglial activation per vascular deposit area so that this apparent increase in staining intensity could not be quantified accurately.

#### Passive amyloid immunotherapy causes increased microhemorrhage

We used the Prussian blue histological stain to label hemosiderin, a ferric oxide material produced in the breakdown of hemoglobin. Extravascular blood in the brain leads to microglial phagocytosis of the erythrocytes and breakdown of the hemoglobin within them. These ferric oxide-containing microglia are thus markers of past hemorrhage. In untreated, aged APP-transgenic mice we observed very few profiles positive for Prussian-blue staining in the frontal cortex (section counterstained with neutral red; Fig. 4A). However, following anti-A $\beta$  antibody treatment for 5 months we observed an increase in the number of Prussian-blue profiles in the frontal cortex, which were readily detectable at a low magnification in the microscope (Fig. 4B). In the absence of anti-A $\beta$  treatment, or even when treated with antibody for one month, most vessels did not stain with Prussian blue, and could be identified only using the red counterstain (Fig. 4C). However, even with 3 months of anti-A $\beta$  antibody treatment we observed frequent vessels with associated Prussian-blue staining (Fig 4D). Using adjacent sections stained for Congo red, we confirmed that all vessels showing microhemorrhage contained amyloid (Figs. 4E and 4F; we were unable to double-label Prussian blue-stained sections with either Congo red or thioflavine-S). However, only a minority of vessels containing amyloid demonstrated hemorrhage.

When we counted the number of Prussian blue-positive profiles in those animals receiving control antibody there

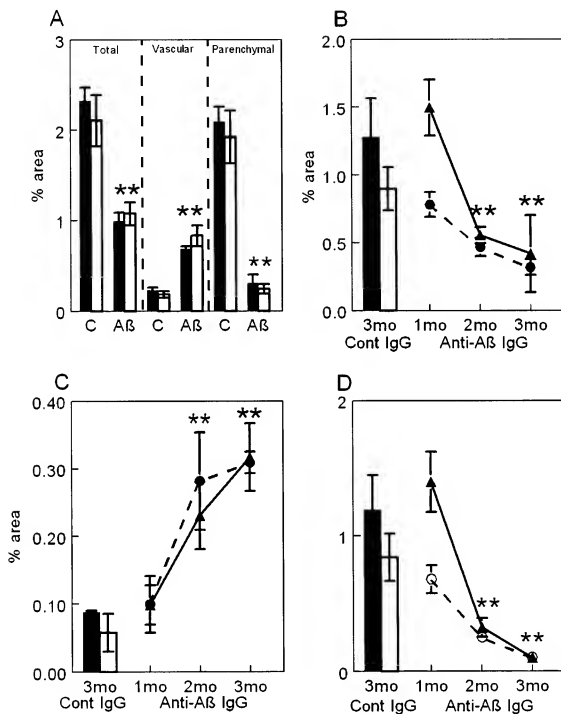
was an average of one profile per every two sections (Fig. 5) and this number remained the same in both control groups (aged 22 or 28 months). Following treatment with anti-A $\beta$  antibody for a period of two months we observed a striking increase in Prussian-blue staining, approximately five times that observed in either the control group or the mice immunized for one month (Fig. 5,  $p < 0.001$ ). Following this initial increase in Prussian-blue staining, we observed a linear increase in staining associated with increasing duration of anti-A $\beta$  antibody treatment (Fig 5). Five months of anti-A $\beta$  antibody treatment demonstrated a six-fold increase in Prussian-blue staining when compared the control groups (Fig. 5).

#### Discussion

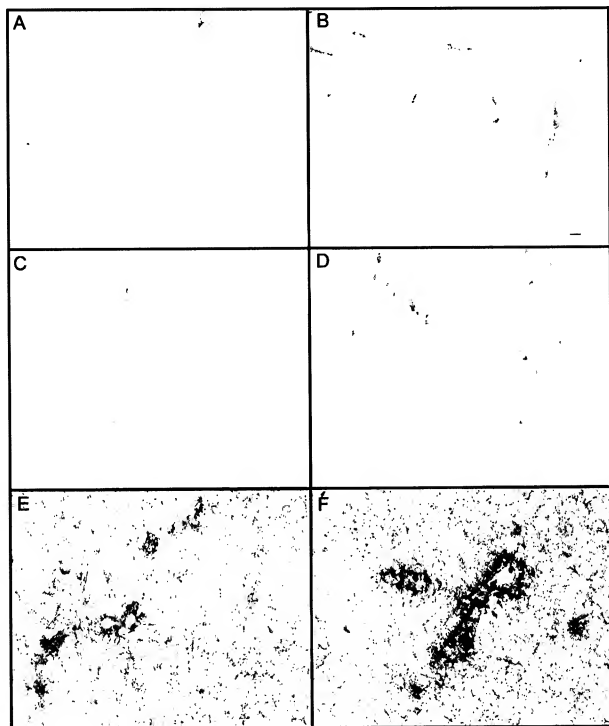
Earlier studies with vaccines against the A $\beta$  peptide demonstrated protection from the learning and memory deficits associated with amyloid accumulation in APP-transgenic mice [14,19]. Passive immunization protocols with anti-A $\beta$  antibodies also produced cognitive benefits, in some cases even in the absence of significant reduction in amyloid burden [12,13]. Our recent work found that 3 months of anti-A $\beta$  treatment of 18-month-old APP-transgenic mice improved spontaneous alternation performance on the Y-maze [14]. In the present work we confirmed that passive anti-amyloid immunotherapy can reverse spatial learning deficits in APP-transgenic mice and that this benefit of immunotherapy is retained, even in aged mice (26 and 28 months old at testing) with long-established amyloid pathology.

Additionally, we describe a more rapid means of testing spatial reference memory to reveal learning and memory deficits in APP-transgenic mice. This two-day version of the radial arm water maze included greater spacing of individual trials (mice spent time in their home cage after every trial), combined with less spacing of aggregate trials (fifteen trials per day rather than four or five) to facilitate learning of platform location in the nontransgenic mice, with a clear absence of learning in the age-matched transgenic mice.

A substantial reduction in total Congophilic amyloid deposits was observed in old APP-transgenic mice treated with anti-A $\beta$  antibodies for 2 or more months. This

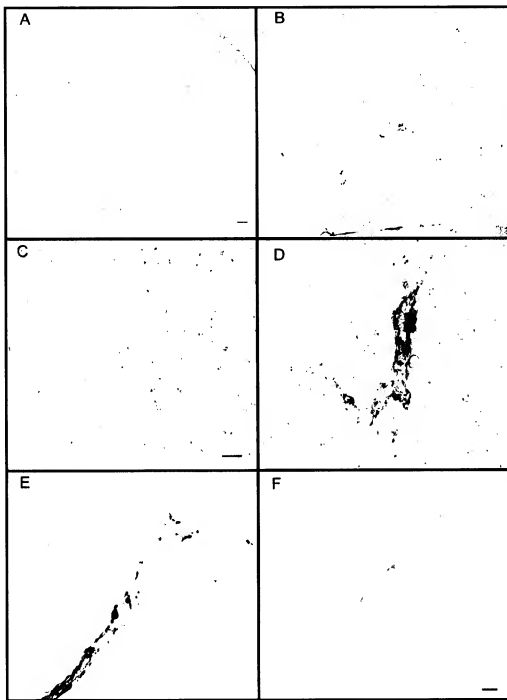
**Figure 2**

Passive immunization with anti-A $\beta$  antibodies decreases total and parenchymal amyloid loads while increasing vascular amyloid in frontal cortex and hippocampus of APP-transgenic mice. Panel A shows total amyloid load measured with Congo red, vascular amyloid load and parenchymal amyloid load from APP-transgenic mice administered control IgG (C) or anti-A $\beta$  IgG (A $\beta$ ) for a period of 5 months. Panels B-D show total amyloid load (Panel B), vascular amyloid load (Panel C) and parenchymal amyloid load (Panel D) from APP-transgenic mice administered control IgG for 3 months (Cont IgG) or anti-A $\beta$  IgG for a period of 1, 2, or 3 months (Anti-A $\beta$  IgG). For all panels, the solid bar and solid line represent values from the frontal cortex, while the open bar and dashed line represent values from the hippocampus. \*\*  $p < 0.01$ .



**Figure 3**

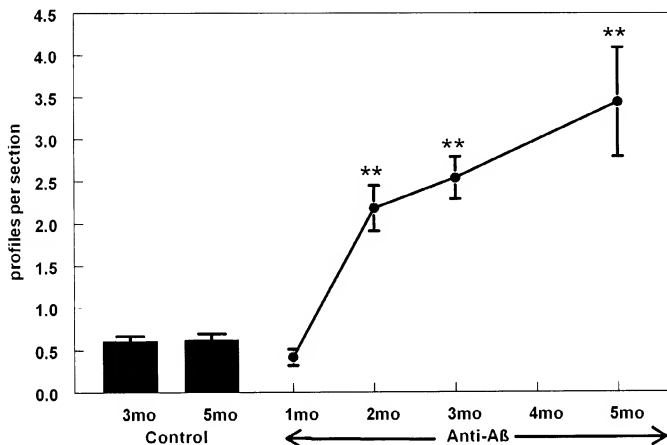
Increased Congo red staining of blood vessels following anti-Aβ antibody administration is associated with activated microglia. Panels A and B are from the frontal cortex of 22-month-old APP-transgenic mice immunized for 3 months with either control antibody (3A) or anti-Aβ antibody (3B). Panels C and D are from the frontal cortex of 28-month-old APP-transgenic mice immunized for 5 months with either control antibody (3C) or anti-Aβ antibody (3D). Panels E and F show a high-magnification image of CD45 immunohistochemistry (black) counterstained with Congo red (red) from 28-month-old APP-transgenic mice immunized for 5 months with either control antibody (Panel E) or anti-Aβ antibody (Panel F). Panels A-D, magnification = 100X. Scale bar in Panel B = 50 μm for panels A-D. Panels E-F, magnification = 200X. Scale bar in Panel E = 25 μm for panels E-F.



**Figure 4**

Microhemorrhage associated with CAA following systemic administration of anti-A $\beta$  antibodies. Panels A and B are low magnification images of the frontal cortex of APP-transgenic mice receiving either control antibodies (Panel A) or anti-A $\beta$  antibodies (Panel B) for a period of 5 months. Panels C and D show representative images of amyloid containing vessels stained for Prussian blue (blue), counterstained with neutral red (red), from APP-transgenic mice receiving either control antibodies (Panel C) or anti-A $\beta$  antibodies (Panel D) for a period of 3 months. Panel E shows a blood vessel in the frontal cortex stained for Prussian blue (blue), counterstained with neutral red, from an APP transgenic mouse administered anti-A $\beta$  antibodies for 5 months. Panel F shows the same blood vessel on an adjacent section stained for Congo red, indicating that the blood vessel does in fact contain amyloid. Scale bar panel A = 120  $\mu$ m for panels A-B. Scale bar panel C = 25  $\mu$ m for panels C-D. Scale bar in panel F = 25  $\mu$ m for panels E-F.





**Figure 5**

Number of Prussian blue-positive profiles increases with duration of anti-A $\beta$  antibody exposure. The graph shows quantification of the average number of Prussian blue-positive profiles per section from mice administered control IgG for 3 or 5 months (Cont) or anti-A $\beta$  IgG for 1, 2, 3 or 5 months (anti-A $\beta$ ). \*\*  $p < 0.01$ .

measurement of total Congo-red staining included both parenchymal and vascular amyloid staining. When we analyzed the sections for only vascular amyloid (CAA) we found that this measure was significantly increased following 2, 3 and 5 months of anti-A $\beta$  antibody treatment. The remaining parenchymal amyloid load was almost completely eliminated with this antibody approach. Clearly, because total amyloid load was significantly reduced not all amyloid was shifted into the vessels; but, it appears that at least some of the Congophilic material was redistributed to the vasculature. At the present time the mechanism for this redistribution is unclear. However, one possibility is that the microglia associated with the antibody-opsonized amyloid, either by phagocytosis or surface binding, and transported the material to the vasculature, possibly in an attempt to expel it. We and others have shown evidence for microglial involvement in the removal of amyloid using both intracranial anti-A $\beta$

antibody injections [11,21] and systemically administered anti-A $\beta$  antibody treatment [14], as well as *ex vivo* studies [10,22]. Here we also report our impression that microglia surrounding CAA vessels in immunized mice expressed more CD45 than control transgenic mice. This increased expression could be due to either increased expression in the same number of microglial cells or an increased number of microglial cells in these animals. It is feasible that this microglial activation was simply in reaction to the presence of increased amyloid in the blood vessels. However, it is equally likely that microglia activated by the opsonized material migrated to the vessels for disposal of the amyloid.

Cerebral amyloid angiopathy (CAA) is defined as the deposition of congophilic material in meningeal and cerebral arteries and arterioles (capillaries and veins can also show CAA but less frequently), and it occurs to some extent in

nearly all Alzheimer's disease patients [23]. Severe CAA, affecting about 15% of cases, can be associated with both infarction and hemorrhagic injury [24,25]. It has also been shown that the severity of CAA can be directly linked to the severity of dementia in Alzheimer's disease patients [26].

In the current study we found a significantly increased number of microhemorrhages in the brain as detected by Prussian-blue staining, associated with the increase in CAA following passive immunization. Another transgenic mouse model of amyloid deposition, the APP23 mice, have been shown to deposit amyloid in both brain parenchyma and blood vessels and show a CAA associated increase in spontaneous cerebral hemorrhages [27]. Moreover, Pfeifer *et al.* [16] showed that these spontaneous hemorrhages were significantly increased following 5 months of passive immunization of 21-month-old APP23 mice using an anti-A $\beta$  antibody with an N-terminal epitope, similar to those typically developed in active immunization with vaccines [4,28,29]. When young mice (6 months of age) were immunized following the same protocol, no hemorrhages were observed. More recently, DeMattos *et al.* [30] showed that passive immunization with an N-terminal antibody (3D6: directed against amino acids 1–5 of A $\beta$ ) of PDAPP transgenic mice also resulted in significantly increased microhemorrhage. They were unable to detect increased microhemorrhage with a mid-domain antibody (266: directed against amino acids 13–28 of A $\beta$ ). Notably, antibody 266 fails to bind A $\beta$  deposited in CAA vessels or amyloid plaques [31]. Importantly, Ferrer *et al.* [7] noted the presence of CAA and microhemorrhage in the brain of one patient that participated in the A $\beta$ -vaccine trial, even though the parenchymal amyloid appeared lower than expected. Also, Nicoll *et al.* [6] noted that CAA appeared unaffected in the brain of another patient that participated in the A $\beta$ -vaccine trial.

It remains to be determined whether these observations regarding increased CAA and microhemorrhage in transgenic mice are relevant to trials of passive immunotherapy in humans. It should be noted that, in spite of extending the period of immunotherapy to 5 months, there was no discernable loss of the cognitive benefits of immunotherapy in the transgenic mice, all of whom showed increased microhemorrhage. While the observation that antibody 266 does not result in vascular leakage encourages testing of this idiomotype, data from the Zurich cohort of the A $\beta$  vaccine trial argue that brain-reactive antibodies may be important for cognitive benefits [32].

## Conclusions

Our opinion is that these results suggest that passive immunotherapy against A $\beta$  should proceed with appropriate precautions taken to minimize the risk of hemor-

rhage (e.g., by excluding patients taking anticoagulants) and instituting measures to detect such hemorrhages if they do occur, irrespective of the antibody specificity or proclivity for microhemorrhage in aged APP-transgenic mice.

## List of abbreviations

A $\beta$ : Amyloid-beta.

APP: Amyloid precursor protein

CAA: Cerebral amyloid angiopathy.

IgG1: Immunoglobulin G type 1.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

DMW treated the mice, performed the behavioral analysis, processed the tissue and performed pathological analyses, and drafted the manuscript. ARojiani evaluated slides and provided expert opinion regarding CAA and microhemorrhage. ARosenthal and SS developed, produced and purified the antibodies used in the studies. MJF performed DNA extraction and PCR for genotyping of the mice. MNG oversees the breeding colony generating mice for the studies, collected samples from the mice and assisted in editing the manuscript. DM conceived the design of the study, guided data interpretation and assisted in editing the manuscript.

## Acknowledgements

This work was supported by National Institutes of Aging / NIH grants AG15490 (MNG) and AG18478 (DMW). DMW is the Benjamin Scholar in Alzheimer's disease research. We would like to thank Keisha Symmonds who aided in histological processing of the tissue and Nedda Wilson who was responsible for animal husbandry during the study. We would also like to thank Lori Lutz for assisting in editing the manuscript.

## References

- Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y: Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* 1994, 13:45-53.
- Gordon MN, Holcomb LA, Jantzen PT, DiCarlo G, Wilcock D, Boyett KW, Connor K, Melachroinou J, O'Callaghan JP, Morgan D: Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1+APP mouse. *Exp Neurol* 2002, 173:183-195.
- Solomon B, Koppel R, Hanan E, Katzav T: Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. *Proc Natl Acad Sci USA* 1996, 93:452-5.
- Schenk D, Barbour R, Dunn W, Gordon G, Grayson D, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandeventer C, Walker S, Yegorov M, Yednock T, Games D, Seubert P: Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999, 400:173-177.
- Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank

- A, Hock C: Subacute meningencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology* 2003, 61:46-54.
6. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO: Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003, 9:448-452.
  7. Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F: Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004, 14:11-20.
  8. Weiskopf ME: Immunology and the elderly: an historical perspective for future international action. *Mech Ageing Dev* 1997, 93:1-6.
  9. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM: Peripheral anti-A $\beta$  antibody alters CNS and plasma A $\beta$  clearance and decreases brain A $\beta$  burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2001, 98:8850-8855.
  10. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberberg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T: Peripherally administered antibodies against amyloid  $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nat Med* 2000, 6:916-919.
  11. Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, Gordon MN, Morgan J: Intracranially administered anti-A $\beta$  antibodies reduce  $\beta$ -amyloid deposition by mechanisms independent of and associated with microglial activation. *J Neurosci* 2003, 23:3745-3751.
  12. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM: Immunization reverses memory deficits without reducing brain A $\beta$  burden in Alzheimer's disease model. *Nat Neurosci* 2002, 5:452-457.
  13. Kotilinek LA, Bacskai B, Westerman M, Kawarabayashi T, Younkin L, Hyman BT, Younkin S, Ashe KH: Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* 2002, 22:6331-6335.
  14. Wilcock DM, Rojiani A, Rosenthal A, Levkowitz G, Subbarao S, Alamed J, Wilson D, Wilson N, Freeman JP, Gordon MN, Morgan D: Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. *J Neurosci* 2004, 24:6144-6151.
  15. Oddo S, Billings L, Kestaji JP, Cribbs DH, LaFerla FM: Abeta Immunotherapy Leads to Clearance of Early, but Not Late, Hyperphosphorylated Tau Aggregates via the Proteasome. *Neuron* 2004, 43:321-333.
  16. Pfeiffer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M: Cerebral hemorrhage after passive anti-A $\beta$  immunotherapy. *Science* 2002, 298:1379.
  17. Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K: Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 1998, 4:97-100.
  18. Gordon MN, King DL, Diamond DM, Jantzen PT, Boyett KV, Hope CE, Hatcher JM, DiCarlo G, Gottschall WP, Morgan D, Arendash GW: Correlation between cognitive deficits and A $\beta$  deposits in transgenic APP+PS1 mice. *Neurobiol Aging* 2001, 22:377-385.
  19. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW: A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000, 408:982-985.
  20. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, George-Hyslop P, Westaway D: A beta peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer's disease. *Nature* 2000, 408:979-982.
  21. Wilcock DM, Munireddy SK, Rosenthal A, Ugen KE, Gordon MN, Morgan D: Microglial Activation Facilitates A $\beta$  Plaque Removal Following Intracranial Anti-A $\beta$  Antibody Administration. *Neurobiol Dis* 2004, 15:11-20.
  22. Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, Guido T, Hoenow K, Hu K, Johnson-Wood K, Khan K, Kholodenko D, Lee C, Lee M, Motter R, Nguyen M, Reed A, Schenk D, Tang P, Vasquez N, Seubert P, Yednock T: Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci USA* 2003, 100:2023-2028.
  23. Jellinger KA: Alzheimer disease and cerebrovascular pathology: an update. *J Neurol Transm* 2002, 109:813-835.
  24. Olichney JM, Ellis RJ, Katzman R, Sabbagh MN, Hansen L: Types of cerebrovascular lesions associated with severe cerebral amyloid angiopathy. *Ann NY Acad Sci* 1997, 826:493-497.
  25. Maurino J, Saposnik G, Lepers A, Rey RC, Sica RE: Multiple simultaneous intracerebral hemorrhages: clinical features and outcome. *Arch Neurol* 2001, 58:629-632.
  26. Thal DR, Ghebremedhin E, Orantes M, Wiestner OD: Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. *J Neuropathol Exp Neurol* 2002, 62:1287-1301.
  27. Winkler DT, Bondolfi L, Herzig MC, Jann L, Calhoun ME, Wiederhold KH, Tolnay M, Staufenbiel M, Jucker M: Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. *J Neurosci* 2001, 21:1619-1627.
  28. Dickey CA, Morgan DG, Kudchodker S, Weiner DB, Bai Y, Cao C, Gordon MN, Ugen KE: Duration and specificity of humoral immune responses in mice vaccinated with the Alzheimer's disease-associated beta-amyloid 1-42 peptide. *DNA Cell Biol* 2001, 20:723-729.
  29. McLaurin J, Cecal R, Kierstead ME, Tian X, Phinney AL, Manea M, French JE, Lambrum MH, Darabie AA, Brown ME, Janus C, Chishti MA, Horne P, Westaway D, Fraser PE, Mount HT, Przybylski M, St George-Hyslop P: Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4-10 and inhibit cytotoxicity and fibrillogenesis. *Nat Med* 2002, 8:1263-1269.
  30. DeMattos RB, Boone IL, Hepburn DL, Parsadanian M, Bryan MT, Ness DK, Pirozi KS, Holtzman DM, Bales KR, Gitter BD, Paul SM, Rakic M: In vitro and in vivo characterization of beta-amyloid antibodies binding to cerebral amyloid angiopathy (CAA) and the selective exacerbation of CAA-associated microhemorrhage. *Neurobiol Aging* 2004, 25(S2):577.
  31. Rakic M, Bryan MT, DeMattos RB: Binding differences between A $\beta$  antibodies in CAA containing vessels from PDAPP-transgenic mice. *Neurobiol Aging* 2004, 25(S2):588.
  32. Hock C, Konietzko U, Streffer JR, Tracy J, Signorini A, Muller-Tillmanns B, Lemke U, Henke K, Moritz E, Garcia E, Wolfler MA, Umbricht D, de Quervain DJ, Hofmann M, Maddalena A, Papassotiropoulos A, Nitsch RM: Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 2003, 38:547-554.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
http://www.biomedcentral.com/info/publishing\_adv.asp



ACCUMULATION OF ANTIBODIES IN THE CENTRAL  
NERVOUS SYSTEM

By JULES FREUND, M.D.

*(From The Henry Phipps Institute, University of Pennsylvania, Philadelphia)*

(Received for publication, March 1, 1930)

The purpose of the present paper is to describe the accumulation of antibodies in the central nervous system of rabbits. Information on this subject may throw light upon several important problems: (1) the penetration of protein (globulin) from the blood into the spinal fluid, brain and cord, for antibodies cannot be separated from globulins experimentally, (2) the relationship between the cerebrospinal fluid and the tissue fluid of the brain and spinal cord and (3) the prevention and treatment of certain diseases of the central nervous system by the injection of immune serums.

The present work is a continuation of a series of studies (1) on the antibody content of the serum and organs of rabbits, which have yielded information as to the distribution of agglutinins in the serum, organs and lymph. Agglutinins against typhoid bacilli were selected as antibodies because they can be obtained in high concentration and can be measured easily and with relative accuracy. The rabbit was employed because the titers of the immune serums of rabbits are very much higher than in other small laboratory animals.\* The experiments were carried out on actively and passively immunized animals. The active immunization consisted of a series of injections of killed typhoid bacilli into an ear vein. The passively immunized rabbits received one injection of immune serum into the blood stream. The antibody content of the organs was measured by extracting the organs (after grinding with sand) with salt solution and titrating the extracts. For the titration of agglutinins a method was used that is more sensitive than the usual routine method of agglutination. This method consists in centrifugalizing the tubes containing the serum or extract-dilutions and bacteria and reading the results while the tubes are being gently shaken and the sedimented bacteria resus-

\* The dog—an animal extensively used in investigations on the central nervous system—does not furnish potent serum. Dogs could have been injected in the passive immunization experiments but by injecting immune serum obtained from rabbits into other rabbits the introduction of foreign protein is avoided.

pended. This technic was first used by Gathgens (2) in 1906 and has been employed by a number of investigators (3) since that time. For further technical details see previous publications of the present series.

The main results of the work, as already reported, can be summarized as follows: When serum containing antibodies is injected into an ear vein of the normal rabbit, antibodies accumulate in all the organs studied: the liver, spleen, kidney, lung, skin, muscle and uterus (smooth muscle). The rate of accumulation varies in the different organs. The final concentration is reached in the liver, spleen, lung and kidney in less than 10 minutes after the injection of immune serum, but in the uterus and skin only after several hours have elapsed. The antibodies penetrate most slowly into the skin. When the final amount of antibodies is accumulated there is a constant numerical relationship between the antibody content of the blood and organs. On the average, the highest dilution of extract prepared from 1 gm. of liver, spleen, kidney, lung or skin that agglutinated typhoid bacilli was one-tenth of the highest dilution of 1 cc. of serum that agglutinated typhoid bacilli.

Since there are less antibodies in the organs than in similar amounts of blood the question naturally arises whether the antibodies recovered from the organs are due to the blood present in them. That they are not derived mainly from the blood of the organs but from the extravascular part of the tissue is evidenced by the following observations. (1) Lymph obtained by cannulating the lymph ducts of the liver, leg, neck and thoracic duct contains antibodies in higher concentration than the organ extracts. (2) Perfusion does not reduce the antibody content of the skin and uterus. (3) More antibodies can be recovered in the perfusate from the living animal than were present in the blood before perfusion, showing that during perfusion antibodies penetrate from the organs into the blood vessels, an observation recently confirmed by Schwarzwann (4).

The equilibrium between the antibody content of the blood and of the organs can be reached from either the blood or the organs, for an identical relationship will establish itself between the antibody content of the serum and organs when the immune serum is injected either into the blood stream or into the skin.

#### EXPERIMENTAL

The experiments to be reported here were performed with both actively and passively immunized rabbits for these reasons. Passive immunization offers an opportunity to establish the rate of accumulation of antibodies in the organs and cerebrospinal fluid by examination of the rabbits at different intervals of time after the injection of immune serum. In actively immunized rabbits the titers of the blood and organ extracts are higher and therefore the observations are more striking. The technic of immunization was the same as in the previous work. The immune serum used was fresh, and was obtained and kept under sterile conditions.

Before describing the experiments, it must be emphasized again that the agglutination tests were made with the aid of centrifugalization, a method that is more sensitive than the routine method of agglutination. Without this technic, the results described in the present study cannot be duplicated.

*I. Antibody-Content of the Brain, Spinal Cord and Cerebrospinal Fluid (from Cisterna Magna) of Actively Immunized Rabbits*

The technic of these experiments differed from that of the earlier experiments in that urethane was not used for anesthesia because it is said that it promotes the

TABLE I  
*Agglutinin Titers of the Serum, Spinal Fluid, Brain and Spinal Cord of Actively Immunized Rabbits*

Number of rabbit	Serum	Spinal fluid	Brain	Cord
1	1:150,000	1:640	1:2,500	1:1,300
2	1:150,000	1:420	1:600	1:384
3	1:128,000	1:160	—	—
4	1:102,000	1:512	1:576	1:1,200
5	1:102,000	1:256	1:1,500	1:362
6	1:102,000	1:512	—	—
7	1:102,000	1:512	—	—
8	1:102,000	1:512	1:583	1:290
9	1:50,000	1:128	—	—
10	1:32,000	1:80	1:200	1:104
11	1:32,000	—	1:192	1:60
12	1:32,000	1:40	—	—
13	1:26,000	1:48	—	—
14	1:20,000	1:52	—	—
15	1:13,000	1:26	—	—
16	1:3,200	1:5	—	—

passage of substances into the spinal fluid. The animals were narcotized with the minimum amount of ether necessary. Some of the rabbits were bled to death from the left carotid artery; some from the femoral arteries and the descending aorta; the site of bleeding did not influence the results. After the rabbits were bled to death cerebrospinal fluid was removed from the cisterna magna by means of a tuberculin syringe and skin-test needle (gauge 22). After some practice there was no difficulty in obtaining fluid free of blood. The samples of cerebrospinal fluid—in most cases 0.4 cc.—were centrifugalized and the sediment examined under a

microscope. Samples containing more than one red blood cell in 10 microscopic fields (seen with high dry lens) were rejected. From the brain and spinal cord the meninges were removed very carefully and the ventricles of the brain and central canal of the cord were then opened and carefully rinsed with salt solution. To ascertain whether any cerebrospinal or other fluid containing agglutinins was left on the surface of the organs, the last washing fluid was examined for agglutinins. Some of the washing fluid caused a trace of agglutination, but dilutions 1 in 2, 1

TABLE II  
*Agglutinin Titers\* of the Spinal Fluid and of Extracts of the Brain and Spinal Cord of Actively Immunized Rabbits*

Number of rabbit	Spinal fluid	Brain extract	Spinal cord extract
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.43	1.70	0.87
2	0.28	0.40	0.26
3	0.12	—	—
4	0.50	0.56	1.17
5	0.25	1.47	0.35
6	0.50	—	—
7	0.50	—	—
8	0.50	0.57	0.28
9	0.26	—	—
10	0.25	0.62	0.32
11	—	0.60	0.18
12	0.12	—	—
13	0.18	—	—
14	0.26	—	—
15	0.20	—	—
16	0.20	—	—
Average.....	0.33	0.82	0.49

\* The titers are expressed as percentages of the titers of the serum.

in 4 did not agglutinate typhoid bacilli. The washed organs were dried by pressing them lightly between filter papers, weighed, and ground with sea sand alone and with saline. The extracts were centrifugalized at high speed, the sediment discarded, and the supernatant fluid centrifugalized again until it became clear. In recording the results of titration, titer 1:100 means that when 1 gm. of brain was extracted with 9 cc. saline, the extract, diluted ten times, clumped typhoid bacilli and a dilution twice higher—1:200—did not agglutinate typhoid bacilli.

Tables I and II show the following.

1. The cerebrospinal fluid of all the rabbits immunized with killed

typhoid bacteria contained antibodies. The titer of the cerebrospinal fluid varied with the titer of the serum, the ratio of the titer of the serum to the titer of the cerebrospinal fluid being, on an average, 300:1, or 0.33 per cent. The variation in this numerical relationship exceeded in one case only the limit of accuracy of the method. One should bear in mind, in this connection, that in the agglutination test the serum, cerebrospinal fluid and organ extract are diluted by halves (1 in 100, 1:200).

2. The brain and the spinal cord of all the actively immunized animals contained agglutinins. The titer of the extracts of the brain and cord varied with the titer of the serums, but the numerical relationship between the titers of the serums and organ extracts was not so consistent as the numerical relationship between the titers of the serums and the cerebrospinal fluid. The ratio of the titers of the serums to the titers of the extracts of the brain and of the spinal cord ranged from 100:0.4 to 100:1.7 and from 100:0.18 to 100:1.17 respectively. The average agglutinin titer of brain extract was 0.82 per cent (of the titer of the serum) and that of the spinal-cord extract 0.49 per cent (of the titer of the serum). Therefore extracts prepared from 1 gm. of brain or spinal cord were more potent than the dilution of a similar amount of cerebrospinal fluid.

Summarizing the results of the observations on actively immunized rabbits, the following average numerical relationship was found between the agglutinin titers of the serum, spinal fluid, brain and spinal-cord extracts:

Blood serum.....	100.	per cent
Cerebrospinal fluid.....	0.33	per cent
Brain extract.....	0.82	per cent
Spinal-cord extract.....	0.49	per cent

## *II. Antibody-Content of the Central Nervous System of Passively Immunized Rabbits*

In studies of the distribution of antibodies in the blood and organs of passively immunized animals it is of great advantage to employ immune serums of high titers; therefore only very potent serums were used for passive immunization. The majority of the animals were injected with immune serums of which the titer was 1:64,000. The titer of one of the immune serums was as high as 1:200,000.



The titer of the serums and the amount injected are given in Table III. The serum was introduced slowly into an ear vein through a skin-test needle (gauge 22), the injection lasting from 3 to 4 minutes. Since it is generally believed that substances in solution injected into a peripheral vein are evenly distributed in the blood stream within 3 minutes, six rabbits were bled to death as early as 15 minutes after the immune serum had been injected. The results of the titrations of the serums, cerebrospinal fluids and organ extracts are tabulated in Tables III and IV.

TABLE III  
*Agglutinin Titers of the Serum, Spinal Fluid, Extracts of Brain and Spinal Cord of Passively Immunized Rabbits*

Number of rabbit	Serum injected		Time between injection of serum and examination	Serum	Spinal fluid	Extract of brain	Extract of spinal cord
	Amount	Titer					
1	10 cc.	1:128,000	15 minutes	1:25,000	0	1:80	1:80
2	10 cc.	1:128,000	15 minutes	1:25,000	0	1:50	1:50
3	25 cc.	1:64,000	15 minutes	1:16,000	1:5	1:96	1:29
4	25 cc.	1:64,000	15 minutes	1:24,000	less than 1:2.5	1:240	1:84
5	15 cc.	1:64,000	15 minutes	1:12,000	1:6	1:264	1:180
6	15 cc.	1:64,000	15 minutes	1:12,000	less than 1:12	—	—
7	10 cc.	1:128,000	2 hours	1:18,000	1:1.8	1:68	1:18
8	10 cc.	1:128,000	2 hours	1:25,000	—	1:120	1:50
9	10 cc.	1:80,000	2 hours	1:10,000	1:6	1:120	1:80
10	25 cc.	1:64,000	3 hours	1:12,000	1:3.6	—	—
11	20 cc.	1:64,000	3 hours	1:12,000	1:32	—	—
12	15 cc.	1:200,000	4 hours	1:32,000	1:19	1:294	1:126
13	20 cc.	1:64,000	18 hours	1:6,400	1:11	1:29	1:10
14	25 cc.	1:64,000	18 hours	1:3,200	1:8	1:13	1:5
15	20 cc.	1:32,000	20 hours	1:3,200	1:3.2	—	—
16	20 cc.	1:64,000	20 hours	1:4,800	1:18	—	—
17	20 cc.	1:64,000	20 hours	1:3,200	1:10	—	—
18	20 cc.	1:64,000	24 hours	1:3,200	1:11.5	—	—
19	20 cc.	1:200,000	24 hours	1:40,000	1:160	—	—

The tables show that in four rabbits, which were bled beginning 10 to 15 minutes after the injection of immune serum and ending 10 minutes later, the undiluted cerebrospinal fluid failed to agglutinate typhoid bacilli. However, in two other rabbits a very small amount of agglutinins penetrated into the cerebrospinal fluid within that time. The penetration of the antibodies into the cerebrospinal fluid (obtained from the cisterna magna) proceeded at a slow rate, for the high-

est titers in the cerebrospinal fluid were found only after 15 hours had elapsed following the injection of immune serum. In these animals the titer of the cerebrospinal fluid ranged between 0.10 to 0.40 per cent of the titer of the serum; that is, it was about as high as in actively immunized rabbits.

TABLE IV  
*Agglutinin Titers\* of the Cerebrospinal Fluid and of Extracts of the Brain and Spinal Cord of Rabbits Following Intravenous Injection of Immune Serum*

Number of rabbit	Time between injection of serum and examination	Titer of cerebrospinal fluid	Titer of brain extract	Titer of extracts of spinal cord
		per cent	per cent	per cent
1	15 minutes	0	0.32	0.32
2	15 minutes	0	0.20	0.20
3	15 minutes	0.03	0.60	0.18
4	15 minutes	less than 0.01	1.00	0.35
5	15 minutes	0.05	2.2	1.5
6	15 minutes	less than 0.1	—	—
7	2 hours	0.01	0.38	0.10
8	2 hours	—	0.48	0.20
9	2 hours	0.06	1.20	0.80
10	3 hours	0.03	—	—
11	3 hours	0.27	—	—
12	4 hours	0.06	0.92	0.43
13	18 hours	0.17	0.45	0.15
14	18 hours	0.25	0.40	0.14
15	20 hours	0.10	—	—
16	20 hours	0.37	—	—
17	20 hours	0.31	—	—
18	24 hours	0.36	—	—
19	24 hours	0.40	—	—

\* The titers are expressed as percentages of the titers of the serums.

Tables III and IV show that just as in actively immunized rabbits, the extracts of the brain and cord of passively immunized rabbits contained agglutinins. The numerical relationship of the titers of the serums and extracts of brain and cord are very similar to those found in the actively immunized animals, the titer of brain extract being on an average 0.70 per cent and that of the cord 0.71 per cent of the titers of the serum. The variation from the average was more marked than in the actively immunized rabbits.

It was expected that the accumulation of antibodies in the central nervous system would proceed at a slow rate. This was found true of the penetration of agglutinins into the cerebrospinal fluid. However it had not been expected that the titers of the organ extracts of rabbits examined 15 minutes after the injection of immune serum would be as high as those from rabbits that had been in contact with immune serum for 24 hours. This unexpected observation suggests either that the penetration of antibodies into the brain and spinal cord (tissue fluid of these organs) is as fast as into some organs, such as the spleen, liver and lung and faster than into the uterus and skin, or that the antibodies recovered were due mainly to the blood present

TABLE V  
*Antibody Titers of Serums and of Extracts of the Perfused Brain of Actively Immunized Rabbits*

Number of rabbit	Titer of serum	Titer of brain extract	Titer* of brain
			<i>per cent</i>
1	25,000	270	1.0
2	25,000	180	0.7
3	12,800	770	0.6
4	250,000	1280	0.5
5	128,000	640	0.5

\* Expressed as percentages of the titers of the serums.

in the brain and spinal cord. To throw light on this question, I perfused the brain of rabbits of which the blood serum had a high titer in the agglutinin test.

### *III. Antibody-Content of the Cerebrospinal Fluid, Brain and Spinal Cord after Perfusion*

The perfusion experiments were performed as follows. An actively immunized rabbit was lightly narcotized with ether, and about 30 cc. of blood was obtained from the left femoral artery. Then 0.15 gm. of heparin was injected into an ear vein; 3 minutes later the rabbit was bled to death from the femoral arteries and the abdominal part of the descending aorta. Immediately after the bleeding was completed, the brachial arteries and descending part of the aorta were ligated, and Locke solution at 42°C. was introduced into the arch of the aorta with the purpose of perfusing the brain through the vertebral and internal carotid arteries. The

perfusion was usually continued for about 1 hour; about 500 cc. of perfusion fluid was used. The examination of cerebrospinal fluid, and of organ extract was carried out as described above.

Table V shows that perfusion did not diminish the antibody titers of the brain extract or the cerebrospinal fluid. However it was felt that before drawing a conclusion from this observation as to the presence of the antibodies in the extra-vascular brain tissue, it was desirable to obtain evidence of the adequacy of the perfusion experiments. To this end I compared histological sections of the brains of the exsanguinated rabbits with those prepared from rabbits whose brain was perfused. The comparison showed that in the unperfused brains the majority of the blood vessels contained red blood cells whereas in the perfused brains the blood vessels were distended and red blood cells were absent from the majority of them.

Final evidence for the view that the antibodies recovered from the brain by extraction are derived from the extra-vascular part of the tissue would be the demonstration of antibodies in the lymph flowing from the brain. However, no lymph duct draining the brain is known; therefore no direct evidence can be furnished at the present time for this view.

#### DISCUSSION

The antibody content of the cerebrospinal fluid of normal and diseased human beings and lower animals has been the subject of extensive clinical and experimental investigations. It has been very generally accepted that antibodies do not penetrate from the blood into the cerebrospinal fluid unless the meninges are inflamed. A survey of the literature, however, shows that several authors have reported the presence of antibodies in the cerebrospinal fluid without inflammation.

Hektoen and Carlson (5) found opsonins but no haemagglutinins in the cerebrospinal fluid of actively or passively immunized dogs. Becht and Greer (6) could not demonstrate agglutinins in the cerebrospinal fluid of rabbits immunized with typhoid vaccine. Kafka (7) found traces of hemolysins and bacterial agglutinins in the cerebrospinal fluid of immunized dogs. Starkenstein and Zitterbart (8) reported that only undiluted cerebrospinal fluid of dogs agglutinated typhoid bacilli, although the titer of their serum was as high as 1:10,000.

There are two reports in the literature on the relative titers of tetanus antitoxin in the serum and cerebrospinal fluid. Ransom (9), working in von Behring's laboratory, compared the antitoxin titers of the serum and of the cerebrospinal fluid of one very highly immunized horse. He found that the ratio of the antitoxin titer of the serum of this animal to that of the cerebrospinal fluid was 100:0.4. Lemaire and Debre (10), who studied the effect of morphine upon the permeability of the meninges, reported that in dogs injected with tetanus antitoxin the ratio of the titer of the serum to that of the cerebrospinal fluid was 100:0.2.

The experiments performed in the present study show clearly that antibodies are present in the cerebrospinal fluid of actively or passively immunized animals without inflammation of the meninges. The possible objection that the antibodies demonstrated were due to inflammation or contamination of the cerebrospinal fluid with blood as a result of faulty technic can be met as follows. (1) The specimens of cerebrospinal fluid did not contain red blood cells at all or only in a negligible number. (2) There was a constant numerical relationship of the titers of serums to the titers of cerebrospinal fluids. (3) In passively immunized rabbits the antibody titer of the cerebrospinal fluid increased during the first 15 hours following the injection of immune serum, although the titer of the blood decreased during this time.

It is pertinent to inquire whether the central nervous system of the rabbits used in these experiments was free of pathological changes. McCartney (11) at the suggestion of Flexner, examined the brains of a large number of apparently healthy rabbits and found histological evidence of meningo-encephalitic lesions in more than 50 per cent. His observation is in conformity with those made in other laboratories (Bull (12), Oliver (13)).

The brains of rabbits employed in the present work were not examined histologically, but it is reasonable to assume that the lesions found by McCartney occur in the brains of our stock rabbits.

McCartney, Bull and Oliver did not study the spinal fluid of their rabbits and therefore it is not known whether the lesions found by them in the brain are associated with a large number of leucocytes or with other signs of inflammation in the cerebrospinal fluid. Although the spinal fluid of the rabbits employed in the present work was free from an abnormal number of leucocytes, the possibility cannot be excluded that meningo-encephalitic lesions so prevalent in apparently normal

rabbits did not influence the accumulation of antibodies in the central nervous system in some of them. However antibodies were found in the spinal fluid, brain and spinal cord in all of the actively and all of the passively immunized rabbits 2 hours after passive immunization. It hardly seems possible that the accumulation of antibodies in the central nervous system of all the rabbits could have been due solely to the presence of meningo-encephalitis.

The reasons for the general belief that antibodies do not penetrate from the blood into the cerebrospinal fluid without the inflammation of the meninges are probably the following. (1) The antibody titer of the cerebrospinal fluid is relatively low, as would naturally follow from the circumstance that the antibody titers of the serums of animals examined in the course of the reported studies were not high. (2) A large number of studies dealt with normal hemolysins, whose titer in the serum is very low.

Amoss and Eberson (14) reported that "agglutinins were not found in the spinal fluid of normal monkeys which had received antimeningococcic serum intravenously." This observation can be readily explained by the experiments on rabbits reported here, which show a ratio of 300:1 between the titers of the serum and of the spinal fluid; whereas the serum of the monkey in the experiment of Amoss and Eberson contained only 100 units of agglutinins per cubic centimeter, and the amount of agglutinins present in the spinal fluid was, therefore, too small to be detected. Flexner, Clark and Amoss (15) found that "it is unusual for the neutralizing principles to be contained in the cerebrospinal fluid during convalescence from epidemic poliomyelitis," although neutralizing principles are present in the blood. It is, however, not probable that the serums of the convalescents contained the neutralizing principles or antibodies in quantities that could be demonstrated in three-hundred-fold dilution.

Although, as my experiments show, antibodies penetrate from the blood into the spinal fluid of rabbits even without inflammation, the fact remains that the antibody content of the spinal fluid is very small. The results of numerous clinical and experimental observations have shown the effectiveness of sterile inflammation and of injecting immune serum into the spinal fluid in raising the antibody content of the spinal fluid (Flexner). In experiments to be reported later this finding has been reobtained in rabbits.

Since antibodies are found in the globulin fraction of the serum, behave in many respects like globulins and may be expected to follow the distribution of globulins, it is interesting to compare the ratio of the antibody titers of the serum and the cerebrospinal fluid on

the one hand with the ratio of the globulin of the serum and that of the cerebrospinal fluid on the other hand. Mestezrat (16) stated that the globulin content of the cerebrospinal fluid is about 0.019 gm. per 100 cc., and it is said that 100 cc. of serum contains on an average 2.5 gm. of globulin. These data were obtained from the serum and cerebrospinal fluid of man, but it is possible that the globulin content of the serum and cerebrospinal fluid in the rabbit is at least of similar magnitude if not almost equal to that found in human beings. The ratio of globulin content of the serum to that of the cerebrospinal fluid,

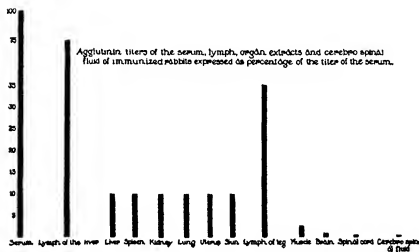


CHART 1

100:0.76, nearly equals that of the respective antibody titers, 100:0.30. Considering the accuracy of the technic of agglutination tests a closer agreement can hardly be expected. Therefore it can be said there is some parallelism between the antibody titers of the serum and cerebrospinal fluid on the one hand and the globulin content of these fluids on the other hand.

These observations have an obvious bearing on the serum therapy of the central nervous system. They show that antibodies do accumulate in the tissue of the brain, spinal cord and cerebrospinal fluid even if the immune serum is not injected into the central nervous sys-

tem; furthermore, that the antibody content of the central nervous system can be estimated by titration of the blood.

It is important to ascertain whether antibodies accumulate in the tissues of the central nervous system at a faster rate when the immune serum is injected into the spinal fluid instead of the peripheral blood stream. This question is being investigated and will be the subject of a subsequent publication.\*

In the previous studies it was found that the agglutinin titers of the extracts of the spleen, liver, lung, kidney, uterus and skin are about the same and on the average ten times lower than that of the serum (extract of 1 gm. of organ compared with dilution of 1 cc. of serum). The titer of extracts of the muscles of the leg is lower than those of the other organs examined, varying from 1 to 5 per cent expressed as percentage of the titer of the serum. In contrast to these organs the brain and cord yield extracts that contain antibodies in very low titer, less than 1 per cent of the titer of the serum. (See Chart 1.)

#### CONCLUSIONS

1. Antibodies can be extracted from the brain and spinal cord of rabbits actively or passively immunized with typhoid bacilli.
2. The titers of the antibodies in the extracts of brain and cord depend upon the titer of the blood serum. In actively immunized rabbits the following numerical relationships exist between the titers of the serum and of these organ extracts: The ratio of the titer of the serum is to the titers of extract of brain and of the spinal cord about as 100 is to 0.8; the titer of the serum is to the titer of the cerebrospinal fluid as 100 is to 0.3. In passively immunized rabbits the titer of the serum is to the titer of brain and spinal-cord extract as 100 is to 0.7.
3. The antibodies recovered from the brain are not due to the presence of blood in it for perfusion of the brain does not reduce its antibody content appreciably.
4. Antibodies penetrate into the spinal fluid from the blood even in the absence of inflammation of the meninges. When the penetration is completed the following numerical relationship exists between the titer of the serum and that of the cerebrospinal fluid: 100 to 0.25.

\* More general discussion of the literature will be published in a subsequent paper.



5. The penetration into the cerebrospinal fluid of antibodies injected intravenously proceeds at a slow rate, being completed only several hours after the immune serum has been injected. The penetration of antibodies into the tissue of the brain occurs at a very rapid rate. It is completed within 15 minutes.

6. It is very unlikely that when the immune serum is injected intravenously the antibodies reach the brain tissue by way of the cerebrospinal fluid, for (1) the antibody titer of the cerebrospinal fluid is lower than that of the brain extract, and (2) antibodies penetrate faster into the tissue of the brain than into the cerebrospinal fluid.

## REFERENCES

1. Freund, J., *J. Immun.*, 1927, 14, 101.  
Freund, J., and Whitney, C. E., *Ibidem*, 1928, 15, 369; 1929, 16, 109.  
Freund, J., *Ibidem*, 1929, 16, 275, 515; 1930, 18, 325.
2. Gathgens, W., *Munch. Med. Woch.*, 1906, 53, 1351; *Arch. f. Hyg.*, 1908, 66, 377.
3. Gates, F. L., *J. Exp. Med.*, 1922, 35, 63.  
Levine, P., and Mabee, J., *J. Immun.*, 1923, 8, 425.  
Freeman, G. C., and Whitehouse, A. J., *Am. J. Med. Sci.*, 1926, 172, 664.  
Mudd, S., *J. Immun.*, 1927, 13, 113.  
Freund, J., see reference No. 1.
4. Schwarzmänn, L. A., *Zeitschr. f. Immunitätsf.*, 1929, 62, 256.
5. Hektoen, L., and Carlson, A. J., *J. Inf. Dis.*, 1910, 7, 319.
6. Becht, F. C., and Greer, J. R., *J. Inf. Dis.*, 1910, 7, 127.
7. Kafka, V., *Zeitschr. f. d. ges. Neurol. u. Psychiatrie*, 1912, 13, 192.
8. Starkenstein, E., and Zitterbart, R., *Wien. klin. Woch.*, 1918, 31, 1317.
9. Ransom, F., *Zeitschr. f. physiol. Chemie*, 1900, 31, 282.
10. Lemaire, J., and Debre, R., *J. de physiol. et de pathol. gen.*, 1911, 13, 233.
11. Flexner, S., *J. Am. Med. Assn.*, 1923, 81, 1688, 1785.  
McCartney, J. E., *J. Exp. Med.*, 1924, 39, 51.
12. Bull, C. G., *J. Exp. Med.*, 1917, 25, 557.
13. Oliver, J., *J. Inf. Dis.*, 1922, 30, 91.
14. Amoss, H. L., and Ebersson, F., *J. Exp. Med.*, 1913, 29, 597.
15. Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, 19, 205.  
Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, 25, 499.  
Flexner, S., *J. Am. Med. Assn.*, 1913, 61, 447, 1872.  
Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1916, 23, 683; 1917, 25, 525.  
Flexner, S., and Ebersson, F., *J. Exp. Med.*, 1918, 27, 679; 1918, 28, 11.
16. Mestezrat, W., *Le liquide cephalo-rachidien normal et pathologique*, A. Maloine, Paris, 1912.

## A $\beta$ N-terminal-end specific antibody reduced $\beta$ -amyloid in Alzheimer-model mice

Yuko Horikoshi<sup>a,b</sup>, Takashi Mori<sup>c</sup>, Masahiro Maeda<sup>b</sup>, Nōriaki Kinoshita<sup>b</sup>,  
Kumiko Sato<sup>a</sup>, Haruyasu Yamaguchi<sup>a,\*</sup>

<sup>a</sup> Gunma University School of Health Sciences, Maebashi, Gunma 371-8514, Japan

<sup>b</sup> Immuno-Biological Laboratories Co., Ltd. (IBL), Fujioka, Gunma 375-0005, Japan

<sup>c</sup> Institute of Medical Science, Saitama Medical Center/School, Kawagoe, Saitama 350-8550, Japan

Received 18 September 2004

Available online 28 October 2004

### Abstract

Alzheimer's disease (AD) is a neurodegenerative disease with memory dysfunction that is causing serious medical problems in modern society. For the fundamental treatment of AD, an amyloid  $\beta$  protein (A $\beta$ ) vaccine is considered to be the most potent candidate. To cure AD, we developed A $\beta$  N-terminal-end specific monoclonal antibody named 82E1, which does not cross-react with full-length A $\beta$  precursor. Passive intraperitoneal administration of 82E1 markedly reduced total plaque area (A $\beta$  burden) in the Tg2576 mouse brains. This was confirmed by the ELISA measurement of insoluble A $\beta$  in the brain homogenates. The density of diffuse plaques, which increases in the late stage, was markedly reduced by the administration of 82E1, suggesting that the reduction of the A $\beta$  burden was due to the prevention of newly developed diffuse plaques. Above results provide an insight into the further therapeutic intervention in AD with few adverse effects.

© 2004 Elsevier Inc. All rights reserved.

**Keywords:** A $\beta$ ; Administration; Alzheimer; Antibody; APP; ELISA; Immunohistochemistry; Tg2576

Alzheimer's disease (AD) is one of the most serious neurodegenerative diseases. Deposition of amyloid  $\beta$  protein (A $\beta$ ), a major component of senile plaque amyloid, promotes neurofibrillary changes and synaptic loss, resulting in dementia in AD. In 1999, Schenk et al. [1] have reported that vaccination with A $\beta$ 1-42 effectively reduced cerebral  $\beta$  amyloid deposition in A $\beta$  precursor (APP) transgenic mouse brains. The clinical trial with A $\beta$ 1-42 (AN-1792) was discontinued due to the adverse effect, namely neuroinflammation [2]. In a subsequent paper, intraperitoneal injection of A $\beta$  antibody has also reduced  $\beta$  amyloid deposition in the transgenic mice, showing the significant effect of passive immunization [3,4]. Since the antibodies used in the above studies

reacted with full-length APP as well as A $\beta$ , the administration of these antibodies would have potential adverse effects in clinical applications. Thus, it is accumulating interest to reduce adverse effects by further modification of A $\beta$  antibodies.

Recently, we developed a monoclonal antibody, named 82E1. It has high specificity and high reactivity against A $\beta$  N-terminal-end, and therefore does not cross-react with full-length APP [5]. Here, we examined the effect of passive immunization with 82E1 on Tg2576 mouse brains.

### Materials and methods

All animal protocols were in accordance with the guidelines of the Animal Use Ethics Committee of the Saitama Medical Center/School

\* Corresponding author. Fax: +81 27 220 8946.

E-mail address: [yamaguchi@health.gunma-u.ac.jp](mailto:yamaguchi@health.gunma-u.ac.jp) (H. Yamaguchi).

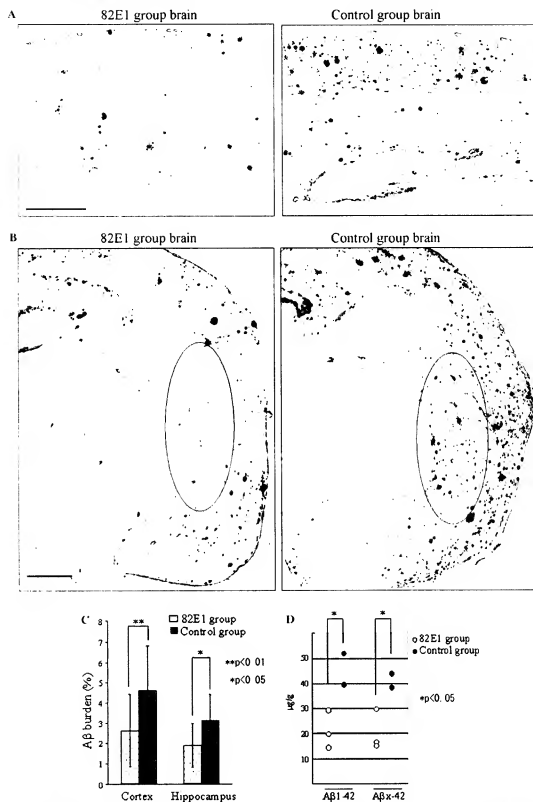


Fig. 1. (A) Aβ burden was reduced by administration of 82E1 antibody when compared to that in the control group; Aβ42 staining. Bar = 500 μm. (B) Senile plaques in basal ganglia (in the circle) were absent in the 82E1 group; Aβ42 staining. Bar = 500 μm. (C) Aβ burden (Aβ42 positive plaque area) of the cortex ( $p < 0.01$ ) and hippocampus ( $p < 0.05$ ) was significantly lower in the 82E1 group than in the control group. (D) Insoluble Aβ1-42 and Aβx-42 were significantly reduced in the 82E1 group based on ELISA measurements. \* $p < 0.05$ , \*\* $p < 0.01$ .

and NIH guidelines [DHHS publication No. (NIH) 85-23, revised 1985].

Purified A $\beta$ 1 N-terminal-end specific monoclonal antibody 82E1 (1 mg/ml in PBS) was administered once a week by intraperitoneal injection at a dose of 10 mg/kg to 16-month-old Tg2576 mice (82E1 group,  $n = 3$ ), while PBS was administered for the control group (Tg2576 mice (control group,  $n = 2$ ) [3]. After 12 times administrations for over 3 months, blood sample was collected transcardially for the ELISA measurement of soluble A $\beta$  in the plasma level under the deep anesthesia by sodium pentobarbital (25 mg/kg), and mice were euthanized by transcardial perfusion of 100 ml of 10 U/ml heparin in saline. The left cerebral hemisphere was fixed in 4% buffered formaldehyde solution and embedded in paraffin. Serial sections (5- $\mu$ m in thickness) of the left cerebral hemisphere at 10 predetermined coronal planes separated by 1-mm intervals were sequentially cut and immunostained with A $\beta$ 40 and A $\beta$ 42 end specific polyclonal antibodies (2  $\mu$ g/ml, IBL, Japan) after brief formic acid pretreatment using an ABC Elite kit (Vector, USA). Additionally, to examine the distribution of 82E1 into the brain parenchyma, one mouse in the 82E1 group was intraperitoneally administered by the biotinylated 82E1 1 day before sacrifice. The distribution of biotinylated 82E1 was visualized using an avidin-horseradish peroxidase in 4% buffered formaldehyde-fixed vibratome sections. Microglia in paraffin sections were visualized using Iba-1 antibody (2  $\mu$ g/ml; Wako, Japan).

Images of 10 selected sections at fixed intervals from the cortex and five from hippocampus of each mouse were acquired using an Olympus BX60 microscope with an attached digital camera system (DP-50, Olympus, Japan), and the digital image was routed into a Windows PC for quantitative analysis using SimplePCI software (Coplax, Imaging Systems, USA). A $\beta$ 40 and A $\beta$ 42 burdens were presented as the percentage of immunolabeled area captured (positive pixels) divided by the full area captured (total pixels).

TBS-insoluble A $\beta$  was extracted using 6 M-guanidine HCl [6] from the right frontal quarter of brains and then used for the ELISA measurement together with plasma. A $\beta$ 1-40 and A $\beta$ 1-42 were measured by combinations of 82E1 with A $\beta$ 40 or A $\beta$ 42 C-terminal end-specific antibodies 1A10 and 1C3, respectively, as described previously [5]. A $\beta$ 1-40 and A $\beta$ 1-42 were also measured by combinations of 12B2, A $\beta$  middle portion antibody, with 1A10 or 1C3 [5].

## Results and discussion

We observed the effect of 82E1 administration. Immunohistochemistry for A $\beta$ 42 showed a smaller number of plaques in the 82E1 group than in the control group (Figs. 1A and B). In the basal ganglia, where A $\beta$  deposit occurred later than that in the cortex and hippocampus, senile plaques were markedly less in the 82E1 group than in the control group (Fig. 1B). This reduction was confirmed by image analysis (Fig. 1C). The A $\beta$  burden was significantly reduced in the 82E1 group compared with that in the control group for both A $\beta$ 40 (30% reduction,  $p < 0.05$ , in cortex; 40% reduction,  $p < 0.05$ , in hippocampus) and A $\beta$ 42 (43% reduction,  $p < 0.01$  in cortex; 40% reduction,  $p < 0.05$ , in hippocampus) (Fig. 1C).

A $\beta$  ELISA analysis of TBS-insoluble fraction was consistent with the findings on immunohistochemical studies. Significant reduction of A $\beta$ 42 was found in the 82E1 group. The 82E1 group showed about 50% less A $\beta$ 1-42 and A $\beta$ x-42 than the control group (Fig. 1D). A $\beta$ 40 tended to reduce in the 82E1 group compared to that in the control group, although its reduction did

not reach a significant level (data not shown). The A $\beta$  level in plasma was not significantly different between the 82E1 and control groups (data not shown).

Although we attempted to visualize the distribution of biotinylated 82E1 antibody into the brain parenchyma, the antibody employed in this study failed to access into the brain parenchyma (data not shown).

From 15 to 23 months in the Tg2576 brains, A $\beta$  deposits increased markedly in number and burden, as shown in the control group [7]. Numbers of diffuse plaques rapidly increase in this period. However, the 82E1 group brains showed fewer diffuse plaques than we expected. Thus, we concluded that the 82E1 administration reduced senile plaques, especially diffuse plaques, in Tg2576 mice.

Bard et al. [4] suggested that clearance of the A $\beta$  deposition was induced by phagocytosis of activated microglia, and that activation of microglia was mediated by Fc receptor. In our study, activated microglia were similarly found in both the 82E1 and control groups (data not shown).

These findings suggest that antibody administration reduced the A $\beta$  burden without microglial activation. In fact, the A $\beta$  clearance mechanism in the brain was independent of microglial activation [8,9]. We could not confirm the distribution of 82E1 antibody into the brain parenchyma. Therefore, our findings agree with the report that the A $\beta$  antibody exists in the peripheral blood and reduces the A $\beta$  burden by altering the central nervous system and plasma A $\beta$  clearance [10].

The current study demonstrated that administration of antibody against the N-terminal of A $\beta$ , like 82E1, effectively reduces plaques. The 82E1 does not react with full-length APP. In the brain, APP exists in neuronal cells, thus an antibody that reacts with full-length APP might bind to normal cells and promote inflammation. Thus, 82E1 might be a good therapeutic agent.

## Acknowledgments

H. Yamaguchi was supported by a Grant-in-Aid for Scientific Research (16300110) and that for Priority Areas (16015101) from the Ministry of Education, Science, Sports, Culture and Technology, Japan, and Life Science Research Foundation of Japan.

## References

- [1] D. Schenk, R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, Z. Liao, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandever, S. Walker, M. Wogulski, T. Yednock, D. Games, P. Seubert, Immunization with amyloid- $\beta$  attenuates Alzheimer-disease-like pathology in the PDAPP mouse, *Nature* 400 (1999) 173–177.

- [2] E. Check, Nerve inflammation halts trial for Alzheimer's drug, *Nature* 415 (2002) 462.
- [3] F. Bard, C. Cannon, R. Barbour, R.L. Burke, D. Games, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, I. Lieberburg, R. Motter, M. Nguyen, F. Soriano, N. Vasquez, K. Weiss, B. Welch, P. Seubert, D. Schenk, T. Yednock, Peripherally administered antibodies against amyloid  $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease, *Nat. Med.* 6 (2000) 916–919.
- [4] F. Bard, R. Barbour, C. Cannon, R. Carretto, M. Fox, D. Games, T. Guido, K. Hoenow, K. Hu, K. Johnson-Wood, K. Khan, D. Kholodenko, C. Lee, M. Lee, R. Motter, M. Nguyen, A. Reed, D. Schenk, P. Tang, N. Vasquez, P. Seubert, T. Yednock, Epitope and isotype specificities of antibodies to  $\beta$ -amyloid peptide for protection against Alzheimer's disease-like neuropathology, *Proc. Natl. Acad. Sci. USA* 100 (2003) 2023–2028.
- [5] Y. Horikoshi, G. Sakaguchi, A.G. Becker, A.J. Gray, K. Duff, P.S. Aisen, H. Yamaguchi, M. Maeda, N. Kinoshita, Y. Matsuoaka, Development of  $A\beta$  terminal end-specific antibodies and sensitive ELISA for  $A\beta$  variant, *Biochem. Biophys. Res. Commun.* 319 (2004) 733–737.
- [6] M. Morishima-Kawashima, N. Oshima, H. Ogata, H. Yamaguchi, M. Yoshimura, S. Sugihara, Y. Ihara, Effect of apolipoprotein E allele  $\epsilon 4$  on the initial phase of amyloid  $\beta$ -protein accumulation in the human brain, *Am. J. Pathol.* 157 (2000) 2093–2099.
- [7] T. Kawarabayashi, L.H. Younkin, T.C. Saido, M. Shoji, K.H. Ashe, S.G. Younkin, Age-dependent changes in brain, CSF, plasma amyloid  $\beta$  protein in the Tg2576 transgenic mouse model of Alzheimer's disease, *J. Neurosci.* 21 (2001) 372–381.
- [8] B.J. Baeska, S.T. Kajdasz, M.E. McLellan, D. Games, P. Seubert, D. Schenk, B.T. Hyman, Non-Fc-mediated mechanisms are involved in clearance of amyloid- $\beta$  in vivo by immunotherapy, *J. Neurosci.* 22 (2002) 7873–7878.
- [9] D.M. Wilcock, G. DiCarlo, D. Henderson, J. Jackson, K. Clarke, K.E. Ugen, M.N. Gordon, D. Morgan, Intracranially administered anti- $A\beta$  antibodies reduce  $\beta$ -amyloid deposition by mechanisms both independent of and associated with microglial activation, *J. Neurosci.* 23 (2003) 3745–3751.
- [10] R.B. DeMattos, K.R. Bales, D.J. Cummins, J.-C. Dodart, S.M. Paul, D.M. Holtzman, Peripheral anti- $A\beta$  antibody alters CNS and plasma  $A\beta$  clearance and decreases brain  $A\beta$  burden in a mouse model of Alzheimer's disease, *Proc. Natl. Acad. Sci. USA* 98 (2001) 8850–8855.

## Brief Communication

## Reversible Memory Loss in a Mouse Transgenic Model of Alzheimer's Disease

Linda A. Kotilinek,<sup>1</sup> Brian Bacskaï,<sup>2</sup> Marcus Westerman,<sup>1</sup> Takeshi Kawarabayashi,<sup>3</sup> Linda Younkin,<sup>3</sup> Bradley T. Hyman,<sup>2</sup> Steven Younkin,<sup>3</sup> and Karen H. Ashe<sup>1</sup><sup>1</sup>Departments of Neurology and Neuroscience, University of Minnesota, Minneapolis 55455, <sup>2</sup>Department of Neurology, Massachusetts General Hospital East, Charlestown, Massachusetts 02129, and <sup>3</sup>Department of Neuroscience, Mayo Clinic, Jacksonville, Florida 32224

Alzheimer's disease (AD) is a neurodegenerative condition, believed to be irreversible, characterized by inexorable deterioration of memory and intellect, with neuronal loss accompanying amyloid plaques and neurofibrillary tangles. In an amyloid precursor protein transgenic mouse model, Tg2576, little or no neuronal loss accompanies age-related memory impairment or the accumulation of A $\beta$ , a 40–42 aa polypeptide found in plaques. Recently, we have shown inverse correlations between brain A $\beta$  and memory in Tg2576 mice stratified by age (Westerman et al., 2002). Broadening the age range examined obscured this relationship, leading us to propose that small, soluble assemblies of A $\beta$  disrupt cognitive function in these mice. Here we show that memory loss can be fully reversed in

Tg2576 mice using intraperitoneally administered BAM-10, a monoclonal antibody recognizing the N terminus of A $\beta$ . The beneficial effect of BAM-10 was not associated with a significant A $\beta$  reduction, but instead eliminated the inverse relationship between brain A $\beta$  and memory. We postulate that BAM-10 acts by neutralizing A $\beta$  assemblies in the brain that impair cognitive function. Our results indicate that a substantial portion of memory loss in Tg2576 mice is not permanent. If these A $\beta$  assemblies contribute significantly to memory loss in AD, then successfully targeting them might improve memory in some AD patients.

**Key words:** Alzheimer's disease; transgenic; behavior; A $\beta$ ; monoclonal antibodies; memory

The Tg2576 transgenic mouse model of Alzheimer's disease (AD), which overexpresses a mutant form of amyloid precursor protein (APP), APP<sub>K670N/M671L</sub>, linked to early onset familial AD, develops amyloid plaques and progressive cognitive deficits (Hsiao et al., 1996). In these mice, A $\beta$  begins to rise rapidly at ~6 months, coincident with the appearance of detergent-insoluble A $\beta$  (Kawarabayashi et al., 2001), and memory ability declines progressively thereafter (Westerman et al., 2002). Punctate, cored plaques are present in 7- to 8-month-old mice; mature, diffuse plaques appear at 12 months of age (Kawarabayashi et al., 2001). Descriptive characterizations of the relationship between memory and A $\beta$  in Tg2576 mice (Westerman et al., 2002), along with active A $\beta$  immunization studies in Tg2576 and other APP transgenic mice (Janus et al., 2000; Morgan et al., 2000), have demonstrated that A $\beta$  is necessary and sufficient to disrupt memory and have implicated a soluble A $\beta$  assembly rather than the accumulation of A $\beta$  or amyloid plaques per se.

There have been no studies addressing whether the deleterious effects of A $\beta$  on cognitive function are permanent. Tg2576 mice at 16 months of age with mature plaque deposition show no neuronal or synaptic loss (Irizarry et al., 1997), leading us to surmise that cognitive impairment in these mice might be attributable to neuronal dysfunction rather than neuronal degeneration. Based on previous studies of the relationship between A $\beta$  and memory in Tg2576 mice (Westerman et al., 2002), we hypothesized that if cognitive deficits related to toxic A $\beta$  assemblies occur primarily in the absence of structural damage, then passive administration of antibodies to A $\beta$  might rapidly reverse learning and memory deficits by neutralizing one or more critical A $\beta$  species, thereby restoring normal cognitive function. To focus our evaluation on alterations in cognitive function that occur before plaque deposition, we tested Tg2576 mice at 9–11 months after the appearance of detergent-insoluble A $\beta$ , but preceding the accumulation of abundant mature amyloid plaques (Kawarabayashi et al., 2001). At this age, punctate deposits are present but are rare and difficult to quantify meaningfully. Because passive immunization affects molecular targets more rapidly and selectively than active immunization, we chose passive immunization as a tool to clarify the molecular mechanism by which memory loss occurs in Tg2576 mice.

## MATERIALS AND METHODS

**Mice and behavioral testing.** Forty-three female Tg2576 mice, positive for the HuAPP695<sub>K670N</sub>/M671L transgene in a hybrid C57BL/6JL background (Hsiao et al., 1996), were longitudinally tested twice at 9–11 months of age: a total of 17 Tg2576-positive mice (10 female, 7 male) were longitudinally tested at 2 and 8 months of age, and 10 littermates negative for the transgene (7 female, 3 male) were tested at 3 months of

Received April 1, 2002; revised May 10, 2002; accepted May 20, 2002.

This work was supported by National Institutes of Health Grants AG15453 (K.H.A., B.T.H., S.Y.), NS33249 (K.H.A.), MH65465 (K.H.A.), and AG08087 (B.T.H.), by a Pioneer Award from the Alzheimer's Association (B.T.H.), and by The Walter Family Foundation (B.T.H.). We gratefully acknowledge Stefanie Schriump, Deirdre Cooper-Blacketer, Jennifer Perry, Anton Gunimares, Jennifer Lang, Jennifer Paulson, and Nardina Nash for their expertise and dedication testing mice in the water maze. We thank Megan McLellan and Steve Kojdas for technical assistance with immunohistological procedures and Eugene Gnida for technical help performing ELISAs.

Correspondence should be addressed to Karen H. Ashe, Department of Neurology, Mayo Mail Code 295, 420 Delaware Street Southeast, Minneapolis, MN 55455. E-mail: ashe001@mayo.edu.

T. Kawarabayashi's present address: Department of Neurology, Okayama University Graduate School of Medicine, Okayama, 700-8558 Japan.

Copyright © 2002 Society for Neuroscience 0270-6474/02/226331-05\$15.00/0

age, in the reference memory version of the Morris water maze (Morris, 1984), as described previously (Westerman et al., 2002).

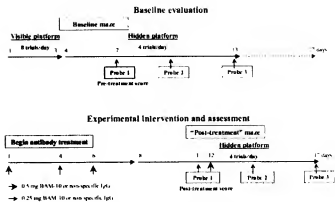
In the longitudinal experiment involving 9- to 11-month-old mice, a baseline assessment of the cohort was obtained immediately before immunization, first in the visible-platform version of the water maze (3 d, eight trials per day) followed by hidden-platform testing (9 d, four trials per day). The spatial memory for the platform position was evaluated in 1 min probe trials administered at the beginning of days 4, 7, and 10 of hidden platform testing. Mice were allocated to the two treatment groups that were counterbalanced on the basis of the mean of the three baseline probe scores. All cues were changed, and the platform position was shifted to the opposite quadrant during subsequent retesting of immunized mice performed 11–12 d after the termination of the baseline water maze test. Only a hidden-platform version of the water maze test was performed. The order of testing mice from different experimental groups was random, and the experimenters were unaware of the treatment group. Eight mice that were unable to learn the visible-platform or became out of the pool with an escape score were removed from the experiment, a proportion consistent with previous studies (Westerman et al., 2002). One mouse died during baseline testing, before immunization, and another mouse died 1–2 hr after the final BAM-10 injection, reducing the final control (IgG) and treatment (BAM-10) group sizes to 17 and 16, respectively. The latter mouse showed no signs of illness at the time of injection, making it likely that the acute death was related to a traumatic injection rather than to encephalitis.

Seventeen naive Tg2576 mice, along with 10 transgene-negative littermates, were also tested at 2 and 3 months of age, respectively, using the same protocol, except that these mice were prehabilitated before testing. Prehabilitation consisted of performing preparative maneuvers resembling procedures used during testing 8–10 times during the 2–3 weeks before actual testing. Previous cross-sectional studies of spatial reference memory during the lifetime of Tg2576 mice in the C57BL/6J background have shown no differences between Tg2576 mice at <6 months of age and nontransgenic littermates at >20 months of age (Westerman et al., 2002). For this reason, we chose to compare Tg2576 mice at 9–11 months of age with younger Tg2576 mice and nontransgenic littermates. At 8 months of age, the 17 Tg2576 mice were allocated into two treatment groups counterbalanced on the basis of mean probe scores at 2 months of age and gender, treated with BAM-10 or non-specific IgG, retested in the water maze beginning at 8.3 months of age, and killed at 8.7 months of age.

**Antibody selection and administration.** BAM-10 (Sigma, St. Louis, MO) is a mouse monoclonal antibody recognizing Aβ(1–12). BAM-10 was chosen on the basis of its ability to bind Aβ *in vivo*. Because not all antibodies bind to Aβ in its native configuration (B. Baeckai and B. T. Hyman, personal communication), we used multiphoton microscopy, an *in vivo* imaging method with ~1 μm resolution, to evaluate the effectiveness of BAM-10 antibody in living Tg2576 mice. We purified, concentrated, and labeled BAM-10 with fluorescein, and applied 5–10 μl of a 1 mg/ml solution directly to the cortical surface of 25-month-old Tg2576 mice. We then visualized the fluorescence as described previously (Baeckai et al., 2001), readily imaging both senile plaques in the neuropil and amyloid angiopathy in the living mouse brain. The *in vivo* immunofluorescent signal colocalized with thioflavin S staining in core plaques and in amyloid angiopathy, as well as revealing nonthioflavin S diffuse deposits (data not shown). Diffuse but not core deposits were reduced by 53% after 3 d in BAM-10-treated mice, an effect similar to that obtained using another antibody recognizing the N terminus of Aβ, 10D6 (Baeckai et al., 2001) (data not shown).

Antibodies were administered intraperitoneally beginning 4–5 d after the last day of baseline water maze pretesting in the longitudinal experiment involving 9- to 11-month-old mice, and 7 d before water maze testing in the experiment involving 8-month-old mice. One group of Tg2576 mice received BAM-10 ascites lacking sodium azide preservative; the other group if mice received IgG (Sigma). Animals received 0.5 mg of antibody on days 1, 6, and 12 and received 0.25 mg of antibody on day 4. Mice were killed on the last day of behavioral testing, 5 d after the last dose.

**Serum antibody titers.** BAM-10 serum titers were measured using an adaptation of methods described previously (Sebenik et al., 1999). Microtiter 11 SA plates (Costar, Cambridge, MA) were coated with 1 μg of aggregated Aβ42 (American Peptide Company, Sunnyvale, CA) in PBS, pH 8.5, and blocked with 1% BSA (Sigma) in PBS, pH 7.4. Plates were washed with wash buffer (PBS, 0.05% Tween 20), and threefold serial dilutions (1:50 to 1:1350) of mouse serum in PBS, 1% BSA, 0.05% Tween 20, and 0.02% sodium azide were incubated overnight at 4°C. Plates were



**Figure 1.** Longitudinal experimental design using Tg2576 mice to determine whether memory loss, once present, can be restored. Spatial reference memory was measured, using the Morris water maze (Morris, 1984), immediately before and after intraperitoneal administration of BAM-10, a monoclonal antibody recognizing the N terminus of Aβ.

washed and incubated for 1 hr at room temperature in a 1:10,000 dilution of sheep anti-mouse HRP conjugate (Jackson ImmunoResearch, West Grove, PA) in PBS, 0.05% Tween, and 0.1% BSA. Plates were washed and developed with 3,3',5,5'-tetramethylbenzidine (1-Step Slow TMB; Pierce, Rockford, IL). The reaction was stopped with an equal volume of 1 M H<sub>2</sub>SO<sub>4</sub>, and plates were read at 450 nm. Optical densities (ODs) of equivalently diluted normal mouse serum were subtracted from test sera to obtain the net OD. The antibody titer was defined as the dilution of serum yielding a net OD that was 50% of the maximal signal for that specimen.

**AB measurements.** Aβ was measured by ELISA using the 3160 capture antibody described previously (Kawarabayashi et al., 2001).

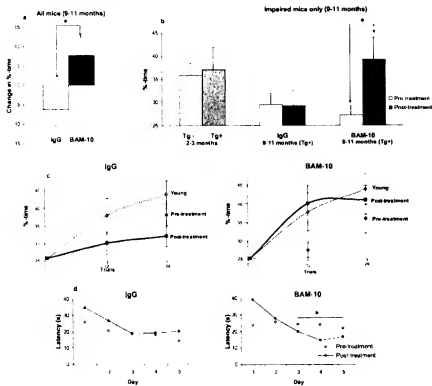
## RESULTS

### BAM-10 restores spatial learning and memory

We measured spatial reference memory, using the Morris water maze (Morris, 1984), immediately before and after treatment with BAM-10 (Fig. 1). Training trials were delivered in blocks of four trials per day, and probe trials were performed on the mornings after the 12th, 24th, and 36th training trials. When memory impairment in Tg2576 mice first emerges at 9–11 months of age, it can be overcome with extensive training, making Tg2576 mice appear to be comparable with nontransgenic littermates at the end of training (Westerman et al., 2002). The slower rate of learning of Tg2576 mice at this particular age is easily detectable at the beginning of training, in the earlier probe scores (Westerman et al., 2002). The first probe scores were therefore used to assess treatment effects in this study.

Animals were assigned to BAM-10 or IgG groups after the baseline maze, counterbalancing for probe scores. The percentage of time spent by mice in the target quadrant during the baseline test in the BAM-10 and IgG groups was not significantly different. Mice received three injections of either 0.5 mg of BAM-10 or non-specific mouse IgG (days 1, 6, and 12 of the experiment) with a booster of 0.25 mg on day 4. BAM-10 serum titers, measured using an adaptation of methods described previously (Schenk et al., 1999), ranged from 1:100 to 1:1100 at 5 d after the last dose. Performance in the water maze was reassessed beginning on day 8. The two groups showed significant differences in changes in performance between baseline and post-treatment tests [ $p = 0.03$  by  $t$  test or by two-way (treatment-by-test session) ANOVA with repeated measures], indicating a significant effect of treatment with BAM-10 (Fig. 2a).

Because not all mice deteriorate at the same rate, a minority of



**Figure 2.** Spatial reference learning and memory in 9- to 11-month-old Tg2576 mice before and after treatment with BAM-10 antibody. The change in retention of spatial memory occurring as a result of receiving BAM-10 or IgG antibodies intraperitoneally was measured by subtracting baseline scores from post-treatment scores to obtain the change in percentage of time spent in the target quadrant (*Change in % time*). *a*, Mice 9–11 months of age receiving BAM-10 antibody showed significantly greater improvement than mice receiving non-specific IgG ( $p = 0.03$  by *t* test; IgG,  $n = 17$ ; BAM-10,  $n = 16$ ). *b*, In mice that were impaired at baseline ( $<40\%$  of the time spent in the target quadrant), those receiving BAM-10 antibody also showed significantly greater improvement than those receiving non-specific IgG ( $p = 0.04$  by two-way ANOVA with repeated measures; IgG,  $n = 13$ ; BAM-10,  $n = 14$ ). Post-treatment performance of impaired mice receiving BAM-10 antibody was significantly higher than baseline performance ( $p = 0.01$  by paired *t* test) and was similar to that of 2-month-old Tg2576 mice ( $n = 17$ ) and 3-month-old nontransgenic littermates ( $n = 10$ ). *c*, BAM-10, but not non-specific IgG, restored the retention learning curve of 9- to 11-month-old Tg2576 mice to resemble that of 2-month-old (Young) Tg2576 mice. *d*, Acquisition of spatial reference memory improved in impaired mice receiving BAM-10 antibody, with significantly reduced mean escape latencies on days 3–5 ( $p = 0.04$  by paired *t* test), but not in mice receiving non-specific IgG. There was a significant treatment-by-training session (baseline vs post-treatment) interaction ( $p = 0.03$  by two-way ANOVA with repeated measures).

9- to 11-month-old mice showed superior performance ( $>40\%$  of time in the target quadrant) comparable with that of the top third of nontransgenic mice. We subsequently segregated the mice on the basis of baseline scores into impaired ( $<40\%$  of time in the target quadrant) and superior ( $>40\%$  of time in the target quadrant) groups. To address the question of whether BAM-10 reversed deficits, we compared the magnitude of the change between baseline and post-treatment scores in impaired mice only. There was a significant treatment-by-test session (baseline vs post-treatment) interaction in impaired mice ( $p = 0.04$  by two-way ANOVA with repeated measures) (Fig. 2*b*). Post-treatment scores in impaired mice receiving IgG showed essentially no change relative to baseline scores. In contrast, impaired mice receiving BAM-10 demonstrated significantly improved scores ( $p = 0.01$  by paired *t* test) (Fig. 2*b*). Remarkably, post-treatment memory ability in BAM-10-treated mice was similar to that of nontransgenic mice tested at 3 months or transgenic mice tested at 2 months, before the onset of memory loss (Fig. 2*b*), indicating that the memory deficits in 9- to 11-month-old Tg2576 mice were reversed and memory was fully restored with BAM-10. These results are supported by comparing the learning curves of retention for BAM-10 and non-specific IgG treatments in Tg2576 mice (Fig. 2*c*). BAM-10 restored the learning curve in 9- to 11-month-old mice to resemble that of 2-month-old Tg2576 mice.

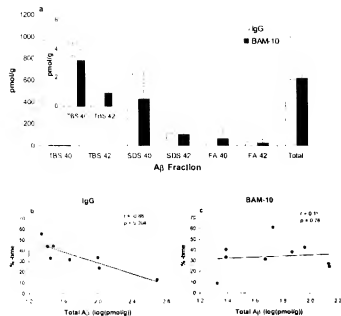
The restorative effects of BAM-10 were also evident when acquisition of spatial reference information was examined in impaired mice. We compared mean escape latencies on days 3–5, because differences in the performance of Tg2576 mice at this age were most pronounced during this phase of training, consistent with the maximal sensitivity of the first probe trial on day 4. There was a significant treatment-by-training session (baseline vs post-treatment) interaction for mean escape latencies ( $p = 0.03$  by two-way ANOVA with repeated measures). Mean escape

latencies measured before and after IgG administration showed no significant differences [mean difference, 2.5 sec; 95% confidence interval (CI),  $-2.8$  to  $7.8$  sec;  $p = 0.32$  by paired *t* test] (Fig. 2*d*). In contrast, mean escape latencies after BAM-10 treatment improved significantly (mean difference,  $-6.3$  sec; 95% CI,  $-0.3$  to  $-12.2$  sec;  $p = 0.04$  by paired *t* test) (Fig. 2*d*). We also observed longer escape latencies on day 1 of the post-treatment test in both groups of mice, suggesting a retest effect in which mice exhibited retention of spatial information from the baseline water maze test. The retest effect rapidly extinguished with retraining in the BAM-10-treated mice but not in the IgG-treated mice. The beneficial effects of BAM-10 were apparent within 11 d of the first antibody dose, the smallest time interval we could measure in this study, given the 8 d elapsing between the first dose and the commencement of retesting in the water maze and the 3 d training interval until the first probe trial.

### No significant changes were observed in A $\beta$ levels

After behavioral testing, the brains of 19 IgG-treated mice and 18 BAM-10-treated mice were sequentially extracted first in Tris-buffered saline (TBS), then in 2% SDS, and finally in 70% formic acid (Kawarabayashi et al., 2001). A $\beta$ 40 and A $\beta$ 42 were then analyzed in each fraction by sandwich ELISA. This analysis showed that the improved performance in mice treated with BAM-10 was not associated with any significant reduction in total A $\beta$  or in A $\beta$ 40 or A $\beta$ 42 in any of the fractions analyzed (Fig. 3*a*). Although BAM-10 was selected on the basis of its ability to bind to and result in the disaggregation of diffuse A $\beta$  deposits in Tg2576 mice when very high concentrations were applied directly to the brain, it is noteworthy that a similar effect on lowering A $\beta$  in the brain was not apparent in mice receiving BAM-10 intraperitoneally.

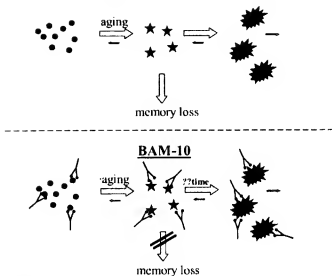




**Figure 3.** Aβ levels in Tg2576 mice treated with BAM-10 or non-specific IgG antibody. Total Aβ is the sum of Aβ40 and Aβ42 in TBS, 2% SDS, and formic acid (F-1) soluble fractions measured as described previously (Kawarabayashi et al., 2001). *a*, Treatment of mice with BAM-10 was not associated with a significant reduction in total Aβ or in Aβ40 or Aβ42 in any of the fractions analyzed (*p* values ranged from 0.2 to 0.9). Measurements represent means ± SDs. Brain Aβ levels were correlated with memory in 8.7-month-old Tg2576 mice treated with BAM-10 or non-specific IgG antibody. *b*, There was a significant inverse correlation between total Aβ and probe scores in control mice treated with non-specific IgG. *c*, Treatment with BAM-10 eliminated the correlation between total Aβ and probe scores.

### BAM-10 eliminates the inverse relationship between Aβ and memory

We have demonstrated previously that there was no obvious relationship between Aβ and memory in Tg2576 mice unless the mice were stratified by age, whereupon significant inverse correlations emerged (Westerman et al., 2002). Because Aβ rises very rapidly between 8 and 12 months of age (Kawarabayashi et al., 2001), tight stratification by age (in days) is necessary to obtain significant correlations between Aβ and memory during this time. To assess the effect of BAM-10 on the relationship between Aβ and memory in Tg2576 mice, we measured brain Aβ and post-treatment spatial reference memory in a second set of mice all exactly 8.7 months of age (all born within 1 d), treated with either BAM-10 or non-specific IgG according to the same schedule as mice in the previously described longitudinal experiment using 9- to 11-month-old mice. As in the previous experiment, treatment with BAM-10 significantly improved memory but had no significant effect on total Aβ or on Aβ40 or Aβ42 in any of the three fractions (data not shown). Analysis of the first probe scores showed a significant negative correlation ( $r = -0.88$ ;  $p = 0.004$  by regression ANOVA) between total Aβ and memory in eight mice treated with non-specific IgG (Fig. 3*b*). A similar negative correlation was observed in these mice for both Aβ40 and Aβ42 in each of the three fractions analyzed, with *r* values ranging from  $-0.70$  to  $-0.87$  and *p* values ranging from 0.005 to 0.05. Because the improved spatial learning and memory in mice treated with BAM-10 was not associated with a significant reduction in Aβ, the negative correlation between Aβ and probe scores was eliminated



**Figure 4.** BAM-10 neutralizes the cognitively disruptive activity of small Aβ assemblies in the brain. *Top*, Memory loss in Tg2576 mice appears to be caused by small Aβ assemblies (stars) (Westerman et al., 2002) formed during the conversion of Aβ monomers (circles) to amyloid deposits (starbursts). Aging refers to the event or series of events occurring as animals age leading to the initial aggregation of monomeric Aβ. Little is known about what comprises these events. *Bottom*, BAM-10 penetrates into the brain, where it may bind to these small Aβ assemblies, neutralize their deleterious effects on cognitive function, and rapidly restore memory in Tg2576 mice. With prolonged treatment, a reduction in amyloid deposits may occur.

by BAM-10 treatment. As shown in Figure 3*c*, there was no significant correlation between Aβ and probe scores in nine mice treated with BAM-10 ( $r = 0.11$ ;  $p = 0.78$  by regression ANOVA).

Although serum BAM-10 titers showed a 10-fold range in levels, there was no correlation between peripheral BAM-10 titers and probe scores ( $r^2 = 0.0002$ ), indicating that the effect of BAM-10 on memory did not depend on blood levels of the antibody. These results suggest that BAM-10 enters the CNS and rapidly neutralizes the deleterious effects of small Aβ assemblies that interfere with cognitive function, thus restoring normal memory in Tg2576 mice.

### DISCUSSION

Active immunization with Aβ was first shown to prevent amyloid deposition (Schenk et al., 1999) and was subsequently shown to prevent cognitive decline (Janus et al., 2000; Morgan et al., 2000) in two APP transgenic models of AD. Passive administration of Aβ antibodies intraperitoneally (Bard et al., 2000) as well as direct application of Aβ antibodies to the brain (Bacskaï et al., 2001) resulted in a reduction of amyloid burden and Aβ levels and a rapid dispersal of deposits. None of these studies addressed the question of whether cognitive deficits, once present, could be restored to normal. Our results show that Aβ antibodies can indeed reverse behavioral deficits in a relatively short period of time. Although the IgG-treated mice performed nearly as well as BAM-10-treated mice after extensive training (data not shown), this does not diminish the observation that learning and memory occurred significantly more slowly in the IgG-treated group.

We postulate that BAM-10, like other Aβ antibodies (Bard et al., 2000), enters the CNS and acts by neutralizing soluble Aβ assemblies disrupting cognitive function. These results support the model we developed to explain the relationship between Aβ

and memory in Tg2576 mice (Westerman et al., 2002) (Fig. 4). Whether the same reversal effect would occur in older Tg2576 mice, where the presumably small amounts of BAM-10 entering the brain would bind to abundant amyloid deposits and therefore might be less available to neutralize soluble A $\beta$  assemblies, is unknown. The direct interaction of BAM-10 with A $\beta$  in the brain is in contrast to the mechanism of action proposed for m266 which, when chronically administered, lowers brain A $\beta$  levels (DeMattos et al., 2001). It has been suggested that m266 exerts its action primarily from outside of the CNS, by creating a peripheral A $\beta$  sink that draws A $\beta$  out of the brain by mass action (DeMattos et al., 2001). We cannot exclude the possibility that BAM-10 exerts a similar indirect effect on brain A $\beta$  in Tg2576 mice. Arguing against this mechanism, however, are the insignificant changes in brain A $\beta$  after BAM-10 administration that are in contrast to the dramatic improvement in memory and the absence of any correlation between serum antibody titers and memory. Whether m266 and BAM-10 operate at distinct sites is an important question to resolve, because whether A $\beta$  antibodies act within or outside of the CNS has important implications for potential inflammatory reactions in human A $\beta$  immunization studies.

The rapid and full restoration of memory suggests that most if not all of the memory impairment in Tg2576 mice at this age occurs by this mechanism, and implies that little if any structural damage is associated with this type of A $\beta$ -mediated brain dysfunction. The possibility that memory loss in humans might be reversed depends on the extent to which the same molecular mechanism that disrupts cognitive function in Tg2576 mice also exists in AD (Klein et al., 2001). Tg2576 mice may represent a model in which memory loss in certain early stages of AD can be studied. If A $\beta$  species that functionally impair normal cognition contribute significantly to Alzheimer's dementia, especially in the early stages, then successfully targeting these species might improve or restore cognitive function.

**Note added in proof.** Rapid reversal of memory loss has also been shown in PDAPP mice receiving passively administered m266 A $\beta$  antibodies (Dodart et al., 2002), which, together with the findings reported here, suggests a common mechanism for memory loss in transgenic APP mice.

## REFERENCES

- Baekai BJ, Kajdasz ST, Christie RH, Carter C, Games D, Seubert P, Schenk D, Hyman BT (2001) Imaging of amyloid- $\beta$  deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med* 7:369–372.
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, et al (2000) Peripherally administered antibodies against amyloid  $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 6:916–919.
- DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (2001) Peripheral anti-A $\beta$  antibody alters CNS and plasma A $\beta$  clearance and decreases brain A $\beta$  burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:8850–8855.
- Dodart JC, Bales KR, Cannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu X, Wu X, Holtzman DM, Paul SM (2002) Immunization reverses memory deficits without reducing brain A $\beta$  burden in Alzheimer's disease model. *Nat Neurosci* 5:452–457.
- Hsiao K, Chapman P, Nilsen S, Eckman C, Hargrave Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, A $\beta$  elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102.
- Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT (1997) APPsw transgenic mice develop age-related A $\beta$  deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* 56:965–973.
- Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D (2000) A  $\beta$  peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408:979–982.
- Kawarabayashi T, Younkin LH, Sado TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid  $\beta$  protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* 21:372–381.
- Klein WL, Krafft GA, Finch CE (2001) Targeting small A $\beta$  oligomers: the solution to an Alzheimer's disease conundrum? *Trends Neurosci* 24:219–224.
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2000) A  $\beta$  peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982–985.
- Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47–60.
- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandever C, et al (1999) Immunization with amyloid- $\beta$  attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–177.
- Westerman M, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, Carlson G, Younkin SG, Ashe KH (2002) The relationship between A $\beta$  and memory in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* 22:1858–1867.

# Effects of $\alpha$ -Synuclein Immunization in a Mouse Model of Parkinson's Disease

Eliezer Masliah,<sup>1,2,\*</sup> Edward Rockenstein,<sup>1</sup> Anthony Adame,<sup>1</sup> Michael Alford,<sup>1</sup> Leslie Crews,<sup>1</sup> Makoto Hashimoto,<sup>1</sup> Peter Seubert,<sup>1</sup> Michael Lee,<sup>3</sup> Jason Goldstein,<sup>3</sup> Tamie Chilcote,<sup>3</sup> Dora Games,<sup>3</sup> and Dale Schenk<sup>3</sup>

<sup>1</sup>Department of Neurosciences

<sup>2</sup>Department of Pathology  
University of California, San Diego  
La Jolla, California 92093

<sup>3</sup>Elan Pharmaceuticals, Inc.  
South San Francisco, California 94080

## Summary

Abnormal folding of  $\alpha$ -synuclein ( $\alpha$ -syn) is thought to lead to neurodegeneration and the characteristic symptoms of Lewy body disease (LBD). Since previous studies suggest that immunization might be a potential therapy for Alzheimer's disease, we hypothesized that immunization with human (h) $\alpha$ -syn might have therapeutic effects in LBD. For this purpose, h $\alpha$ -syn transgenic (tg) mice were vaccinated with h $\alpha$ -syn. In mice that produced high relative affinity antibodies, there was decreased accumulation of aggregated h $\alpha$ -syn in neuronal cell bodies and synapses that was associated with reduced neurodegeneration. Furthermore, antibodies produced by immunized mice recognized abnormal h $\alpha$ -syn associated with the neuronal membrane and promoted the degradation of h $\alpha$ -syn aggregates, probably via lysosomal pathways. Similar effects were observed with an exogenously applied FITC-tagged h $\alpha$ -syn antibody. These results suggest that vaccination is effective in reducing neuronal accumulation of h $\alpha$ -syn aggregates and that further development of this approach might have a potential role in the treatment of LBD.

## Introduction

Lewy body disease (LBD) is a heterogeneous group of disorders that includes Parkinson's disease (PD) and dementia with Lewy bodies (DLB) (Hansen and Galasko, 1992; Kosaka et al., 1984; McKeith, 2000), characterized by degeneration of the dopaminergic system (Shastri, 2001), motor alterations (Braak et al., 2002), cognitive impairment (Salmon et al., 1996), and formation of Lewy bodies (LBs) in cortical and subcortical regions (Trojanowski and Lee, 1998). The number of patients affected by these devastating conditions continues to climb as the population ages, creating a serious public health problem. The cause for LBD is controversial, and multiple factors probably play a role, including various neurotoxins and genetic susceptibility factors (Betarbet et al., 2000; Coleman et al., 1996; D'Amato et al., 1986; Forno et al., 1996; Jenner, 1998; Veldman et al., 1998).

In recent years, new hope for understanding the pathogenesis of this disease has emerged. Specifically, several studies have shown that the synaptic protein  $\alpha$ -synuclein ( $\alpha$ -syn) (Iwai et al., 1994) plays a central role in LBD pathogenesis since (1) this molecule accumulates in LBs (Spillantini et al., 1997; Takeda et al., 1998b; Wakabayashi et al., 1997), (2) mutations and multiplication in the  $\alpha$ -syn gene are associated with rare familial forms of parkinsonism (Kruger et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003), and (3) its expression in transgenic (tg) mice (Lee et al., 2002; Lee et al., 2004b; Masliah et al., 2000) and *Drosophila* (Feany and Bender, 2000) mimics several aspects of PD. Thus, the fact that accumulation of  $\alpha$ -syn in the brain is associated with similar morphological and neurological alterations in species as diverse as humans, mice, and flies suggests that this molecule contributes to the development of LBD.

The mechanisms by which accumulation of  $\alpha$ -syn leads to neurodegeneration and the characteristic symptoms of LBD are unclear. However, recent studies suggest that abnormal accumulation of  $\alpha$ -syn oligomers in the synaptic terminals and axons plays an important role (Hashimoto and Masliah, 1999; Iwatsubo et al., 1996; Lansbury, 1999; Trojanowski et al., 1998).

Although experimental therapies utilizing neurotrophic factors (Kirik et al., 2004) and grafting of dopaminergic cells (Kim, 2004; Yoshizaki et al., 2004) have yielded promising results, alternative approaches directed at reducing the neuronal accumulation of  $\alpha$ -syn are necessary. Viable strategies might include the use of viral vectors expressing antiaggregation molecules (Burton et al., 2003; Hashimoto et al., 2004) or factors such as immunotherapy, which promote the degradation or clearance of  $\alpha$ -syn. For example, recent studies in a tg mouse model of Alzheimer's disease (AD) have shown that antibodies against  $\beta$ -amyloid 1-42 (A $\beta$ ) promote the removal of amyloid from the brain, resulting in improved cognitive performance (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999). Moreover, a vaccination approach has been shown to be effective experimentally in tg mice at reducing the accumulation of prion protein (Sigurdsson et al., 2002) and huntingtin (Luthi-Carter, 2003; Miller et al., 2003), molecules that, like  $\alpha$ -syn, accumulate intracellularly. In this context, we postulate that immunization of human (h) $\alpha$ -syn tg mice (a mouse model of LBD) with purified recombinant h $\alpha$ -syn might activate an immune response that will reduce h $\alpha$ -syn accumulation and, more importantly, neurotoxicity. This approach may be a suitable target for the development of an alternative immune therapy for PD and other disorders with LBs, parkinsonism, and dementia.

## Results

### Characterization of Antibody Titers, Relative Affinity, and Epitope Mapping

Three-month-old tg mice for group I and six-month-old tg mice for group II were immunized for 8 months with

\*Correspondence: emasliah@ucsd.edu

Table 1. Summary of hu-Syn Antibody Titers and Relative Affinity, Corrected for Titer

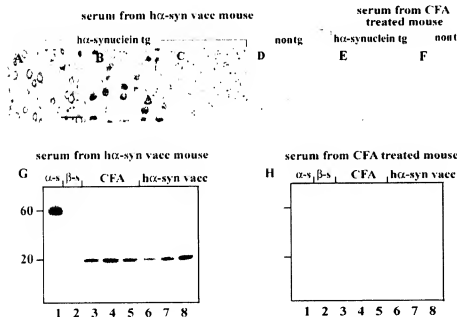
Group	Antibody Relative Affinity by Miniblot	Antibody Affinity to Synapses	Antibody Affinity to Inclusions	Antibody Titers (First Bleed)	Antibody Titers (Second Bleed)	Antibody Titers (Third Bleed)
Group I/hu-syn	109,147 $\pm$ 2700	1.9 $\pm$ 0.73	1.2 $\pm$ 0.4	2332 $\pm$ 500	2772 $\pm$ 1176	3644 $\pm$ 2365
Group I/CFA	113 $\pm$ 113	0.4 $\pm$ 0.1	0	19 $\pm$ 6.7	30 $\pm$ 12	7 $\pm$ 4
Group II/hu-syn	235,747 $\pm$ 74,000	4.1 $\pm$ 0.9	2.8 $\pm$ 1.0	3813 $\pm$ 1200	2926 $\pm$ 976	1468 $\pm$ 641
Group II/CFA	400 $\pm$ 358	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	23 $\pm$ 9	21 $\pm$ 14	0.6 $\pm$ 0.6

recombinant hu-syn or Complete Freund's adjuvant (CFA) alone. Antibody titers in animals belonging to group I ranged from 200 to 20,000 (Table 1), and in mice from group II, titers ranged from 200 to 13,000 (Table 1). Levels of antibody relative affinity by immunoblotting were higher in mice from group II compared to immunized mice from group I (Table 1). By immunocytochemistry (ICC), sera from mice vaccinated with hu-syn showed either labeling of neurons (Figure 1A), intraneuronal inclusions (Figure 1B), or presynaptic terminals (Figure 1C) in hu-syn tg mice, but only displayed mild background staining in nontg mice (Figure 1D). Sera from mice treated with CFA alone showed nonspecific background staining in both hu-syn tg (Figure 1E) and nontg (Figure 1F) mice. By Western blot (WB) analysis, sera from mice vaccinated with hu-syn recognized hu-

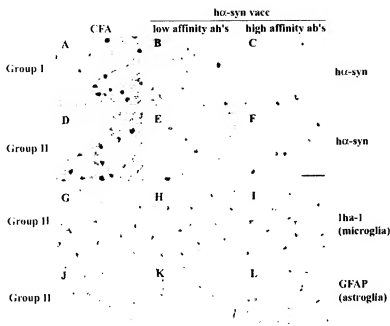
syn in tg mice (Figure 1G). In contrast, no immunoreactivity (IR) was observed with sera from mice treated with CFA alone (Figure 1H). Epitope mapping studies showed that in the vaccinated mice, antibodies recognized epitopes within the C terminus region of hu-syn, including amino acids (aa) 85–99, 109–123, 112–126, and 126–138.

#### Immunization Reduces hu-Syn Accumulation and Preserves Synaptic Density in tg Mice

To determine the effects of immunotherapy and antibody relative affinity on hu-syn accumulation, sections were analyzed by ICC with antibodies against hu-syn (Figure 2). Since antibody relative affinity (Figure 3A) and titers (Table 1) show a wide range of variability and linear regression analysis shows that antibody relative



**Figure 1.** Immunocytochemical and Immunoblot Analysis with the Sera from Vaccinated Mice in the Brains of nontg and hu-Syn tg Mice. For panels (A)–(F), serial vibratome sections from nonimmunized hu-syn tg (Line D, 6-month-old) and nontg mice were immunostained with sera (normalized to 1:1000) from CFA and hu-syn-vaccinated (vacc) animals. All panels are from the temporal cortex. (A)–(C) Sections from hu-syn tg mice immunolabeled with the sera from immunized mice displayed neuronal staining (A), inclusion body staining (B), and granular neuropil immunolabeling suggestive of presynaptic terminals (C). (D) Section from a nontg mouse immunostained with the sera from immunized mice shows only mild background staining. (E and F) Sections from hu-syn tg (E) and nontg (F) mice immunolabeled with sera from CFA-treated mice showed only nonspecific background staining of the neuropil. Scale bar, 40  $\mu$ m. For panels (G) and (H), homogenates from the brains of hu-syn tg mice treated with CFA or immunized with hu-syn were resolved by SDS-PAGE, and blots were probed with the sera from vaccinated mice. (G) Immunoblot analysis with the sera from vaccinated mice shows a prominent band at approximately 19 kDa corresponding to recombinant hu-syn (lane 1, u-s). hu-syn in the brains of tg animals treated with CFA (lanes 3 through 5) or vaccinated with hu-syn (lanes 6 through 8). The sera did not recognize recombinant hu-syn (lane 2, u-s). (H) Immunoblot analysis with the sera from CFA-treated animals shows no IR to recombinant hu-syn (lane 1, u-s), recombinant  $\beta$ -syn (lane 2,  $\beta$ -s) or in the brains of tg animals treated with CFA (lanes 3 through 5) or vaccinated with hu-syn (lanes 6 through 8).



**Figure 2.** Patterns of hu-Syn IR and Glial Response in the Brains of Immunized hu-Syn tg Mice

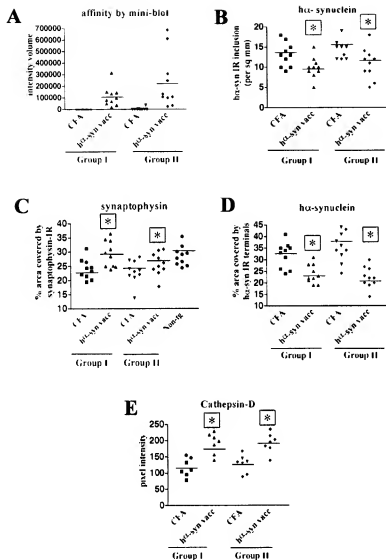
Panels are from the temporal cortex of CFA or hu-syn-vaccinated mice reacted with antibodies against hu-syn, Iba-1, or GFAP, developed with DAB, and analyzed by bright field microscopy. Panels (A)–(C) are representative images from the brains of group I mice (11-month-old), and panels (D)–(L) are from the brains of group II mice (14-month-old). Mice from both groups were divided into subsets of animals that produced antibodies with low (<100,000 units) or high (>100,000 units) relative affinities to hu-syn. (A and D) CFA-treated mice showed abundant hu-syn IR in intraneuronal inclusions as well as the neuropil. (B, E, C, and F) Immunized mice that produce low relative affinity (B and E) antibodies display a modest decrease in hu-syn-immunoreactive inclusions and neuropil labeling, and those that produce high relative affinity antibodies (C and F) demonstrate a more significant decrease in hu-syn-immunoreactive inclusions and neuropil labeling. (G–L) Compared to CFA-treated mice (G and J), animals vaccinated with hu-syn show only a mild increase in microglial IR (Iba-1, [H and I]) and mild astroglial activation (GFAP, [K and L]). Scale bar, 50  $\mu$ m.

affinity was one of the strongest predictors for the effects on reducing the neuropathology on the vaccinated hu-syn tg mice (Table 2), animals were divided into subgroups consisting of those that show low (<100,000 units; Figures 2B, 2E, 2H, and 2K) and high (>100,000 units; Figures 2C, 2F, 2I, 2L, and 3A) relative affinity to hu-syn (expressed as units of intensity volume). In tg mice treated with CFA alone, abundant hu-syn IR was observed in the neuropil as well as in intraneuronal inclusions (Figures 2A and 2D). In contrast, immunized mice from both groups showed a reduction in the number of inclusions in the temporal cortex (Figure 3B). Compared to mice producing low relative affinity antibodies (Figures 2B and 2E), this effect was more pronounced in mice producing antibodies with high relative affinity to hu-syn (Figures 2C and 2F). Moreover, immunization resulted in a decrease in hu-syn IR in the neuropil of tg mice producing antibodies with high relative affinity to hu-syn (Figures 2C and 2F), with a greater effect in mice from group II (Figure 2F) than group I (Figures 2C and 2D), compared to CFA-treated tg mice (Figures 2A and 2D). To investigate whether the effects of the immunization were accompanied by a neuroinflammatory response, immunocytochemical analysis with antibodies against the microglial marker (Iba-1) and the astroglial marker [glial fibrillary acidic protein (GFAP)] were performed. These studies showed that in both CFA-treated (Figures 2G and 2J) and hu-syn-vaccinated (Figures 2H, 2I, 2K, and 2L) animals, there was a mild increase in Iba-1 and GFAP IR; however, no overt differences were detected between the groups.

To further investigate the effects of the vaccination on the synapses, higher-resolution analysis with the laser scanning confocal microscope (LSCM) was performed. These studies confirmed that compared to CFA

controls (Figures 3D, 4A, and 4C) the reduction in the neuropil immunostaining was associated with decreased accumulation of hu-syn in presynaptic terminals (Figures 3D, 4B, and 4D). To ascertain the effects of the immunotherapy on neuropil integrity, sections were immunostained with an antibody against synaptophysin (Figures 4E–4H). Compared to nontg mice, hu-syn tg mice treated with CFA alone (Figures 4E and 4G) showed an average of 20% decrease in the number of synaptophysin-immunolabeled terminals (Figure 3C). In contrast, immunized mice from both groups I and II showed levels of synaptophysin IR comparable to nontg controls (Figures 3C, 4F, and 4H). This effect was more prominent in mice that produced antibodies with high relative affinity to hu-syn (Figure 3C).

To rule out immunization masking effects, control experiments were performed by comparing the levels of murine (m) $\alpha$ -syn. Similar to hu-syn, m $\alpha$ -syn IR was abundant in the neuropil in association with nerve terminals, but was absent from the neuronal cell bodies and in the inclusions (Figures 4I–4L). Both in CFA (Figures 4I and 4K) and hu-syn immunized mice (Figures 4J and 4L), patterns and levels of m $\alpha$ -syn were comparable. Additional analysis of the immunization specificity was performed by comparing levels of  $\beta$ -syn IR, a close  $\alpha$ -syn homolog (Iwai et al., 1994). Abundant  $\beta$ -syn IR was observed in the neuropil in association with the presynaptic terminals, and mild immunolabeling was detected in the neuronal cell bodies, but not in the inclusions (Figures 4M–4P). Compared to tg mice treated with CFA alone (Figures 4M and 4O), no differences in the patterns and levels of  $\beta$ -syn were found in mice immunized with hu-syn (Figures 4N and 4P). These studies suggest that vaccination specifically affects hu-syn but not other related synaptic molecules.



**Figure 3.** Image Analysis of the Levels of hA-syn IR and Other Markers of Neurodegeneration

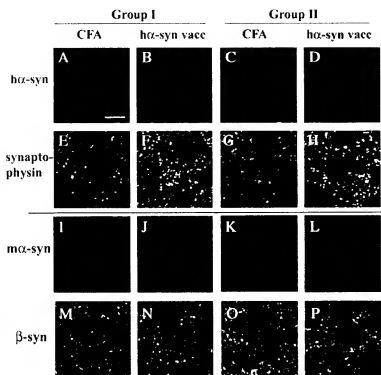
(A) Relative affinity by mini-blot of hA-syn antibodies produced by mice treated with CFA alone or immunized with hA-syn was assessed. (B) Scatterplot of number of hA-syn-positive inclusions in the temporal cortex of mice treated with CFA alone or immunized with hA-syn. Vaccination resulted in a significant decrease in the number of inclusions in both groups compared to controls, an effect more pronounced in group II mice (14-month-old) as opposed to group I (11-month-old). (C) Percent area of the neuropil occupied by synaptophysin-immunoreactive terminals in the temporal cortex. Compared to nontg controls, in tg mice treated with CFA alone, the number of synaptophysin-immunoreactive terminals decreased by an average of 20%. In contrast, tg mice from both groups vaccinated with hA-syn, there was a decrease in the accumulation of hA-syn in synaptophysin-immunoreactive terminals. (D) Percent area of the neuropil occupied by hA-syn-immunoreactive terminals in the temporal cortex. In tg mice from both groups vaccinated with hA-syn, there were significantly higher levels of cathepsin D IR in both groups compared to CFA controls. \* = significant difference compared to CFA-treated hA-syn tg mice ( $p < 0.05$ , unpaired, two-tailed Student's *t* test).

To better characterize the effects of vaccination on hA-syn aggregation in the synapses, double immunocytochemical analysis with antibodies against hA-syn and the presynaptic terminal marker synaptophysin and WB analysis with synaptosomal preparations were performed. Under physiological conditions, hA-syn is localized primarily to the presynaptic boutons (Iwai et al., 1994), and in LBD and the tg mice, increased accumulation of hA-syn in the synapses is associated with functional deficits and synapse loss (Hashimoto et al., 2001). Confocal imaging showed that in comparison to CFA-treated hA-syn tg mice (Figure 3D), vaccinated mice displayed a decrease in the proportion of synaptophysin-immunoreactive nerve terminals in the neocortex that show hA-syn immunolabeling (Figure 3D). Immunoblot analysis with synaptosomal preparations (Figure 5A, upper panel) showed that immunization decreased the accumulation of hA-syn higher molecular weight bands (Figure 5A, upper panel, and Figure 5B), but no effects were observed on mA-syn (data not shown). Furthermore, compared to CFA-treated tg mice,

levels of synaptophysin IR were higher in the synaptosomal preparations from immunized mice (Figure 5A, lower panel, and Figure 5B). These results suggest that immunotherapy might ameliorate the neuronal damage in the brains of tg mice by reducing the accumulation of potentially toxic hA-syn in the synapses.

#### Immunization Effects Are Dependent on the Relative Affinity of Antibodies to Recognize Membrane-Associated Aggregated hA-Syn

Since abnormal accumulation of hA-syn is associated with translocation of hA-syn from the cytosol to the membrane, it is possible that this might explain the antibodies' ability to recognize abnormally accumulated hA-syn in the immunized tg mice. WB analysis showed abundant monomeric hA-syn in both the cytosol (Figure 5C) and membrane (Figure 5D). However, oligomeric hA-syn was more abundant in the membrane fractions where, compared to CFA controls, immunization with hA-syn resulted in decreased accumulation of hA-syn oligomers (Figure 5D).



**Figure 4.** Immunocytochemical Analysis of hα-Syn, Synaptophysin, mα-Syn, and β-Syn IR in the Brains of hα-Syn tg Mice from Groups I and II

All panels are from the temporal cortex of CFA-treated or vaccinated mice. Group I, 11-month-old; group II, 14-month-old. (A–D) Sections were reacted with an antibody against hα-syn, labeled with Tyramide Red and imaged with the LSM. Compared to tg mice treated with CFA alone (A and C), immunized mice (B and D) showed a reduction in the number of inclusions in the temporal cortex as well as a decrease in hα-syn IR (presynaptic terminals) in the neuropil. (E–H) Sections were immunostained with an antibody against synaptophysin and FITC-labeled for imaging with the LSM. In tg mice treated with CFA alone (E and G), levels of synaptophysin IR were reduced, whereas immunized mice showed levels of synaptophysin IR comparable to nontg controls. (I–P) Sections were reacted with an antibody against mα-syn and labeled with Tyramide Red (I–L) or with an antibody against β-syn and FITC-labeled (M–P), and imaged with the LSM. (I–L) Levels of endogenous mα-syn remain unchanged in tg mice treated with CFA alone (I and K) and immunized mice (J and L). All display mα-syn IR in the neuropil, but there is no labeling of the intraneuronal inclusions as is found with antibodies

against hα-syn. (M–P) Both hα-syn tg mice treated with CFA alone (M and O) and tg mice vaccinated with hα-syn (N and P) display abundant β-syn IR in the neuropil and mild immunolabeling in the neuronal cell bodies, but there is no labeling of the intraneuronal inclusions as is found with antibodies against hα-syn. Scale bar, 10 μm.

To better understand which factors might predict the effectiveness of the immunotherapy, linear regression analysis was performed between the neuropathological markers of hα-syn accumulation and the antibody titers and relative affinity (Table 2). A significant correlation was observed between relative antibody affinity by immunoblot and levels of hα-syn IR in the synapses, but not with the numbers of neuronal inclusions. Similarly, relative antibody affinity to recognize synapses by ICC was inversely correlated with levels of hα-syn in the synapses and directly correlated with the percent area occupied by synaptophysin-labeled nerve terminals, but not with the numbers of neuronal inclusions. Antibody titers correlated with the percent area of the neuropil labeled with the anti-hα-syn antibody, but negatively correlated with neuronal inclusions (Table 2). These results suggest that the relative immunoblot reactivity of the anti-hα-syn antibodies and to some extent the antibodies' titers correlate with the reduction of neuronal hα-syn accumulation.

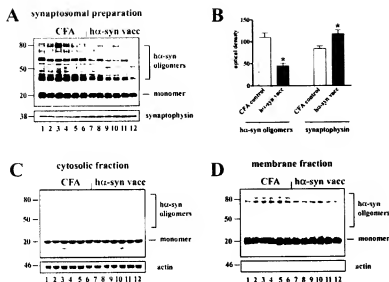
#### Anti-hα-Syn Antibodies Are Internalized and Trigger Clearance of hα-Syn Aggregates via Lysosomal Activation

To determine if anti-hα-syn antibodies generated by the immunized mice recognize hα-syn aggregates in the tg mice, immunocytochemical analysis was performed with horse anti-mouse IgG antibodies. Double immunostaining experiments showed that compared to CFA-treated tg mice (Figures 6A–6C), in hα-syn vaccinated mice, the anti-mouse IgG and the hα-syn IR were colocalized in the periphery of the cell bodies (Figures 6D–6F), the neuritic processes and synapses (Figures 6G–6I). In hα-syn containing neurons, the two markers were detected in granular subcellular structures averaging 0.4–0.8 μm in diameter (Figures 6G–6I). Furthermore, these granular structures displayed cathepsin D IR, suggesting that the internalized anti-hα-syn antibodies reacted with hα-syn within lysosomes (Figures 6M–6O).

To corroborate that vaccination resulted in lysosomal activation, cathepsin D IR was analyzed. Compared to

**Table 2.** Summary of Correlations between Antibody Relative Affinity, Neuropathology, and Titers

Neuropathological Markers	Antibody Relative Affinity by Miniblot	Antibody Affinity to Synapses	Antibody Affinity to Inclusions	Antibody Affinity to Neurons	Antibody Titers (First Bleed)
Number of α-syn (+) inclusions	–0.11	0.04	0.12	–0.21	0.1
% area of neuropil α-syn (+) synapses	–0.46 (p = 0.003)	–0.41 (p = 0.009)	–0.43 (p = 0.005)	0.06	–0.47 (p = 0.007)
% area of neuropil synaptophysin (+) synapses	0.06	0.35 (p = 0.04)	0.01	0.04	0.12
Antibody relative affinity by miniblot	—	0.74 (p = 0.0001)	0.70 (p = 0.0001)	–0.16	0.85 (p = 0.0001)
Antibody titers (first bleed)	0.85 (p = 0.0001)	0.62 (p = 0.0001)	–0.18	0.81 (p = 0.0001)	—



**Figure 5.** WB Analysis of the Levels of hA-syn IR in Synaptosomal, Membrane, and Cytosolic Preparations from Vaccinated Animals

(A and B) Compared to brains of tg mice treated with CFA alone (lanes 1 through 6), in hA-syn-vaccinated tg mice (lanes 7 through 12), levels of hA-syn oligomers were decreased (upper panel), while levels of synaptophysin IR increased in the immunized animals (lower panel). (C and D) Compared to the cytosolic fraction of the brains of tg mice (C), a majority of hA-syn high molecular weight oligomers are present in the membrane fraction (D), where compared to mice treated with CFA alone (lanes 1 through 6), there is a decrease in hA-syn oligomers in the fractions from vaccinated animals (lanes 7 through 12). \* = significant difference compared to hA-syn tg mice treated with CFA alone ( $p < 0.05$ , unpaired, two-tailed Student's *t* test). Animals were 14 months old.

CFA controls (Figures 7A–7C), in vaccinated mice there was increased cathepsin D IR (Figures 3E and 7D–7F). Similar increases in levels of cathepsin D IR were detected in the hA-syn-vaccinated mice from both groups when compared to the CFA-treated group (Figure 3E). In vaccinated tg mice, the cathepsin D-immunolabeled lysosomes were colocalized with discrete hA-syn-immunoreactive granular aggregates (Figure 7F), but no colocalization was observed in CFA-treated tg mice (Figure 7C).

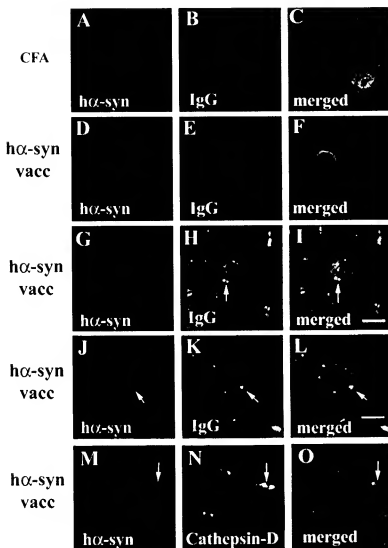
To determine if exogenously applied antibodies recognize hA-syn aggregates in tg mice and activate lysosomal pathways, purified monoclonal anti-hA-syn antibodies were tagged with fluorescein isothiocyanate (FITC) and injected into tg and nontg animals. The FITC-tagged anti-hA-syn recognized hA-syn aggregates in the neuronal cell bodies and synapses of tg animals (Figure 7G–I), but not the endogenous mo-syn in the nontg controls (Figures 7J–7L). Furthermore, in tg mice treated with the FITC-tagged antibody, there was an increase in neuronal cathepsin D IR and colocalization with hA-syn (Figures 7M–7O) compared to nontg controls (Figures 7P–7R). No specific labeling of hA-syn or increase in cathepsin D IR was detected in control experiments where tg and nontg mice were treated with a nonimmune FITC-tagged IgG (data not shown). These studies suggest that circulating antibodies might recognize abnormally aggregated hA-syn associated with the neuronal membrane, which in turn might lead to clearance via lysosomal activation.

## Discussion

The present study showed that hA-syn antibodies generated in vaccinated mice reduced the abnormal accumulation of this protein in the neuronal cell bodies and synapses and ameliorated the loss of synaptophysin-immunoreactive nerve terminals in hA-syn tg mice. This is consistent with recent *in vitro* studies showing that intracellular antibodies can inhibit  $\alpha$ -syn aggregation

(Emadi et al., 2004; Zhou et al., 2004). Moreover, our findings are consistent with studies showing that immunotherapy might reduce the accumulation of extracellularly deposited proteins such as A $\beta$  (Games et al., 2000; Lemere et al., 2003; Morgan et al., 2000) and of intracellular proteins such as huntingtin (Luthi-Carter, 2003), as well as membrane-associated molecules such as prion proteins (Bainbridge and Walker, 2003; White et al., 2003; White and Hawke, 2003). More recently, a study showed that immunotherapy with copolymer-1-immune cells might reduce neurodegeneration in the MPTP model of PD (Benner et al., 2004). While this immunotherapy approach focused on protecting via a general anti-inflammatory mechanism, the present study sought to elicit a specific response to promote degradation of toxic hA-syn. The mechanisms through which the antibodies generated in the vaccinated mice might recognize and promote the clearance of intracellular hA-syn aggregates and other neuronal proteins are less clear. One possibility is that circulating antibodies in the CNS might recognize and cross-link abnormally conformed proteins in the neuronal cell surface (Figure 8). For this, at least a limited amount of the target antigen must be present in the neuronal cell membrane (Luthi-Carter, 2003). A similar mechanism has been described in the antibody-mediated clearance of viruses from cells (Garzon et al., 1999; Ubol et al., 1995). Interestingly, in the case of  $\alpha$ -syn, under basal conditions the monomeric forms of this molecule are almost exclusively present in the cytosolic fraction (Eliezer et al., 2001; Iwai, 2000). In contrast, under pathological circumstances leading to  $\alpha$ -syn aggregation, oligomers and protofibrils can be found in the plasma membrane (Eliezer et al., 2001; Lansbury, 1999; Dixon et al., 2005), where they could potentially be recognized by circulating antibodies. Supporting this possibility, immunoblot and immunocytochemical analysis showed that vaccination reduced hA-syn accumulation in the membrane and that mouse IgG and hA-syn were colocalized in the periphery of the neurons, suggesting





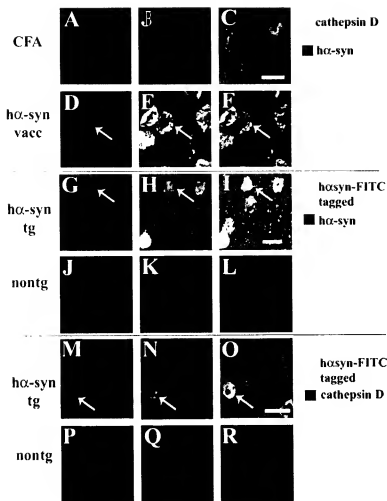
**Figure 6.** Double Immunocytochemical Analysis of the Patterns of *hu-Syn* and anti-IgG or Cathepsin D IR in Vaccinated Animals

All images are from the temporal cortex. Sections from CFA-treated and *hu-syn* vaccinated animals from group II were double labeled with antibodies against *hu-syn* (panels to the left, red) and FITC-tagged anti-mouse IgG or cathepsin D (central panels, green) and imaged with the LSM. The images to the right represent the merged panels (yellow indicates colocalization of the two markers), demonstrating that neuronal cell bodies labeled by the anti-mouse IgG and the intraneuronal granular structures labeled by cathepsin D displayed *hu-syn* IR (arrows). (A–C) Tg mice treated with CFA show characteristic inclusions that react strongly with *hu-syn* antibodies and show background levels of reactivity with anti-mouse IgG. (D–F) Tg mice vaccinated (vacc) with *hu-syn* show a close colocalization between *hu-syn* and anti-mouse IgG in the periphery of the neuronal cell bodies. (G–I) Additionally, the anti-mouse IgG immunostaining is colocalized with *hu-syn* in small granular structures within the cell bodies (arrows). (M–O) These small granular structures also displayed lysosomal cathepsin D IR that colocalized with *hu-syn* immunolabeling. Scale bar, 10  $\mu$ m (A–I), 5  $\mu$ m (J–L). Animals were 14 months old.

that such interactions might take place in the neuronal surface.

Since circulating antibodies might be able to recognize membrane bound  $\alpha$ -syn, there are several possibilities as to how they might promote the clearance of intracellular aggregates. Antibodies in close opposition with the neuronal surface might enter the neurons alone via surface receptors or in association with membrane bound  $\alpha$ -syn and promote lysosomal degradation (Figure 8). This process may be mediated by receptor-dependent or receptor-independent endocytosis of the antibody or antibody-antigen complex, followed by fusion with lysosomes and incorporation of  $\alpha$ -syn aggregates (Figure 8). Supporting this possibility, anti-mouse IgG, an exogenously applied FITC-tagged anti-*hu-syn* antibody, and cathepsin D colocalized with *hu-syn* aggregates. Consistent with the possibility that internalization of the antibody complex might play a role in clearance of  $\alpha$ -syn aggregates, previous studies have shown that neurons can uptake immunoglobulins (Fabian and Petroff, 1987) and internalize some through the

Thy 1.1 receptor in the neuronal and synaptic plasma membrane (Fabian, 1990). Other receptors that might be involved in this process and mediate the endocytosis of antibodies or antibody-antigen complexes include lipoprotein receptor-related protein (LRP) (Herz et al., 1990; Kounnas et al., 1995). This receptor is highly abundant in neurons (Schneider and Nimpf, 2003; Husain, 2001), and macromolecules endocytosed via LRP target the lysosomal pathway (Gonias et al., 2004), suggesting that clearance of intracellular *hu-syn* aggregates might involve lysosomal degradation. In support of this mechanism, the present study showed that in the neurons of vaccinated mice, granular *hu-syn*-immunoreactive structures were also labeled with antibodies against the lysosomal marker cathepsin D. Similarly, previous studies have shown that immunoglobulins internalized by neurons form granular cytoplasmic structures that display lysosomal-like activity (Meeker et al., 1987). Moreover, recent studies have shown that lysosomally dependent autophagy might facilitate the clearance of  $\alpha$ -syn oligomers (but not fibrils) and there-



**Figure 7.** Antibody Recognition of hu-Syn and Lysosomal Activation in Vaccinated hu-Syn tg Animals

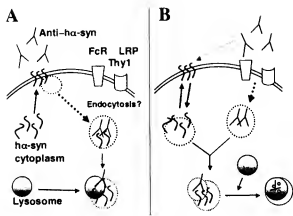
All panels are LSM images from the temporal cortex. Panels (A–F) are from CFA-treated or immunized hu-syn tg mice from group II (14-month-old), and panels (G–R) are from non-immunized nontg and hu-syn tg animals (5-month-old) that received intracerebral injections of an FITC-tagged hu-syn antibody. (A–C) Images from sections of a hu-syn tg mouse treated with CFA alone double immunostained with antibodies against hu-syn (red) and the lysosomal marker cathepsin D (green). (D–F) Images from sections of a vaccinated hu-syn tg mouse double immunostained with antibodies against hu-syn (red) and cathepsin D (green). Yellow indicates colocalization between granular hu-syn-immunoreactive structures and cathepsin D IR (arrows). Note the higher levels of cathepsin D IR in these images. (G–I) Images from sections of a hu-syn tg mouse that received intracerebral injections of an FITC-tagged antibody against hu-syn (green) coimmunolabeled with a different antibody against hu-syn (red). Yellow indicates colocalization between injected and immunolabeled anti-hu-syn antibodies. (J–L) Images from sections of a nontg mouse that received intracerebral injections of an FITC-tagged antibody against hu-syn (green) coimmunolabeled with a different antibody against hu-syn (red). (M–O) Images from sections of a hu-syn tg mouse that received intracerebral injections of an FITC-tagged antibody against hu-syn (green) coimmunolabeled with an antibody against cathepsin D (red). Yellow indicates colocalization between hu-syn and cathepsin D (arrows). (P–R) Images from sections of a nontg mouse that received intracerebral injections of an FITC-tagged antibody against hu-syn (green) coimmunolabeled with an antibody against cathepsin D (red). Scale bar, 15  $\mu$ m.

fore may play a protective role (Cuervo, 2004; Cuervo et al., 2004; Lee et al., 2004a). These studies suggest that in the immunized mice, internalized antibodies might promote degradation of hu-syn aggregates via activation of lysosomal pathways. Alternatively, active vaccination might also promote the clearance of  $\alpha$ -syn aggregates via an immune-mediated cellular response. This is unlikely, because in the present study, no apparent infiltration by lymphocytes was noted and only mild microglial activation was detected. However, it is important to consider this possibility because active immunization in other neurodegenerative disorders has been associated in some cases with vasculitis and autoimmune response (Ferrer et al., 2004), and caution should be exercised when considering the use of active immunization as a potential therapy in patients with PD. For this reason we are currently investigating the effects of passive immunization with antibodies against various regions of  $\alpha$ -syn.

Antibodies against  $\alpha$ -syn not only decreased accumulation in neuronal cell bodies, but also in neuropil, suggesting that the immunotherapy also reduced the accumulation of hu-syn aggregates in the synapses. This effect was associated with amelioration of the neurodegenerative pathology. It is unclear whether this

might be accompanied by the improvement of behavioral deficits; experiments are currently underway to assess this possibility. The ability of the antibodies to recognize neuronal cell bodies and synapses was a good predictor of their ability to reduce hu-syn aggregation and neurodegeneration at least as determined by synaptic integrity. This is consistent with studies in AD, where antibodies with high affinity for plaques displayed a better response (Hock et al., 2002). While for A $\beta$ , the most effective antibodies recognize the N terminus (Bard et al., 2003), for hu-syn, high relative affinity antibodies recognized epitopes within the C terminus. Recent studies have shown that C-terminal fragments of  $\alpha$ -syn assemble into protofibrils and associate with the membrane (McLean et al., 2000; Kim et al., 2002; McLean et al., 2000). Deletion of key amino acids 125–140 within the C-terminal domain greatly alters  $\alpha$ -syn aggregation (Kim et al., 2002), and in the brains of patients with LBD as well as in tg animal models, there is abundant accumulation of C-terminal  $\alpha$ -syn fragments (Iwatsubo et al., 1996; Takeda et al., 2000; Takeda et al., 1998a). These studies suggest that the antibodies' ability to recognize the C-terminal region might be important for the therapeutic effects.

Considerable effort has recently been directed to-



**Figure 8.** Potential Mechanisms of Antibody-hu-Syn Recognition and Targeting to the Lysosomal Pathway

(A) Antibodies might recognize hu-syn bound to the membrane. This complex might in turn be endocytosed and targeted for lysosomal degradation. (B) Receptor-mediated endocytosis of the antibodies and hu-syn aggregates that in turn could be targeted for lysosomal degradation.

ward the development of immunization therapies for neurodegenerative disorders, including the use of active and passive immunization strategies (Frenkel and Solomon, 2001; Morgan et al., 2000; Schenk et al., 1999). PDA vaccination (Luthi-Carter, 2003), and, more recently, adoptive transfer of immune cells to protect nigrostriatal neurons (Benner et al., 2004). Adding to this growing body of evidence supporting a role of immunization in the management of neurodegenerative disorders, the present study suggests that vaccination is effective in reducing the neuronal accumulation of toxic hu-syn aggregates and that further development of this approach might have a potential place in the treatment of LBD.

#### Experimental Procedures

##### Vaccination of hu-Syn tg Mice

For this study, heterozygous tg mice (Line D) expressing hu-syn under the regulatory control of the platelet-derived growth factor- $\beta$  (PDGF- $\beta$ ) promoter (Masliah et al., 2000) were used. These animals were selected because they display abnormal accumulation of detergent-insoluble hu-syn and develop hu-syn-immunoreactive inclusion-like structures in the brain. Although some nuclear staining has been observed in this model, distinct cytoplasmic inclusion-like structures have been consistently identified by confocal and electron microscopy (Masliah et al., 2000; Masliah et al., 2001; Rockenstein et al., 2002). Furthermore, these animals also display neurodegenerative and motor deficits that mimic certain aspects of LBD. Experimental animals were divided into two groups. For the first group, a total of 20 young (3-month-old) tg mice were immunized for 8 months with purified recombinant hu-syn expressed in *E. coli* from sequence-verified hu-syn cDNA ( $n = 10$ ) or CFA alone ( $n = 10$ ). For the second group, a total of 20 young adult (6-month-old) tg mice were immunized for 8 months with recombinant hu-syn ( $n = 10$ ) or CFA alone ( $n = 10$ ). The immunization protocol consisted first of an injection with recombinant hu-syn (80  $\mu$ g/ml; 100  $\mu$ l) with CFA (Sigma-Aldrich, St. Louis, MO). Two weeks later, mice received another injection of hu-syn (80  $\mu$ g/ml; 100  $\mu$ l) with incomplete Freund's Adjuvant (FA), followed by reinjection once a month (for the subsequent 7 months) with hu-syn (80  $\mu$ g/ml; 100  $\mu$ l) in phosphate-buffered saline. Recombinant hu-syn was prepared and purified and tested for endotoxins at Elan following

a modified version of a previously described protocol (Hashimoto et al., 1998).

**Determination of Antibody Titers and Relative Affinity to hu-Syn**  
Antibody levels in plasma were determined using 96-well microtiter plates coated with 0.4  $\mu$ g per well of purified full-length hu-syn. Samples were incubated overnight followed by goat anti-mouse IgG alkaline phosphatase-conjugated antibody (1:7500, Promega, Madison, WI). The plate was read at wavelengths of 450 nm and 550 nm. Results were plotted on a semi-log graph with relative fluorescence units versus serum dilution. Antibody titer was defined as the dilution at which there was a 50% reduction from the maximal antibody binding.

To determine the relative affinity of the antibodies for hu-syn, three assays were performed. In the first, brain homogenates from nonimmunized hu-syn tg mice were run in a minigel, multichannel apparatus (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes. Each channel on the membrane was separated and incubated individually with diluted serum from each of the vaccinated mice. Serum dilution was adjusted to 1:1000 for all samples based on serum titers. Purified recombinant hu-syn was used as a standard, and all channels were normalized to this as a control. Channels were then incubated with secondary rabbit anti-mouse antibody (Vector Laboratories, Burlingame, CA) followed by  $^{125}$ I-tagged protein A (ICN Pharmaceuticals, Costa Mesa, CA) (Alford et al., 1994). Blots were imaged and analyzed with the PhosphorImager (Molecular Dynamics, Piscataway, NJ). For the second, to determine the specificity of the antibodies, brain homogenates from nontg animals, CFA-treated hu-syn tg mice, and immunized hu-syn tg mice were analyzed by WB with serum from CFA-treated mice and high relative affinity serum from hu-syn vaccinated tg mice. Blots were imaged and analyzed on a VersaDoc XL imaging apparatus (BioRad, Hercules, CA). For the third, serial vibratome sections from an untreated hu-syn tg mouse were incubated in diluted serum from each of the treated mice (adjusted to 1:1000) followed by biotinylated horse anti-mouse IgG (1:100, Vector), Avidin D-horseradish peroxidase (HRP, 1:200, ABC Elite, Vector), and reacted with diaminobenzidine tetrahydrochloride (DAB) containing 0.01%  $H_2O_2$ . After microscopic examination, sections were scored according to the cellular compartment labeled (neuronal cell bodies, synapses, and inclusions) and the degree of IR (0 = none; 1 = very mild, 2 = mild, 3 = moderate, 4 = intense).

##### Epitope Mapping of hu-Syn Antibodies

The epitopes recognized by hu-syn antibodies were determined by an ELISA that measures the binding of an antibody to overlapping linear peptides that covered the entire hu-syn sequence. C-terminally biotinylated peptides with sequences of hu-syn (Mimotopes, San Diego, CA) were prepared as 15 aa long peptides with an overlap of 12 residues and a step of 3 residues per peptide. To run the assay, these biotinylated peptides were coated down overnight at 5 nM onto ELISA plates precoated with streptavidin (Pierce, Rockford, IL), followed by washing and incubation for 1 hr with diluted serum samples. Serum samples with titers lower than 5000 were diluted 1:1000 for this incubation. After another washing step, the bound antibodies were detected using species-specific secondary antibodies conjugated to HRP in a colorimetric ELISA format.

##### Tissue Processing

Following NIH guidelines for the humane treatment of animals, mice were anesthetized with chloral hydrate and flush-perfused transcardially with 0.9% saline. Briefly, the right hemisphere was frozen and homogenized for determinations of hu-syn IR by WB (Masliah et al., 2000). The left hemisphere was fixed in 4% paraformaldehyde (PFA) and serially sectioned with the vibratome (Leica, Wetzlar, Germany) for ICC analysis.

##### Synaptosomal Preparation, Membrane and Cytosolic Fractionation, and Immunoblot Analysis

Synaptosomal fractions were prepared essentially as previously described (Dodd et al., 1981a; Dodd et al., 1981b). After resuspension in 0.32 M sucrose, synaptosomal fractions were analyzed by SDS-PAGE on a 10% tris-acetate polyacrylamide gel (NuPAGE, In-

vitrogen) and transferred onto Immobilon membranes (Millipore). For further analysis, total brain homogenates were separated into membrane and cytosolic fractions, prepared essentially as previously described (Hashimoto et al., 2002). The cytosolic and membrane fractions were analyzed by SDS-PAGE on 4–12% Bis-Tris gels and transferred onto Immobilon membranes (Millipore).

Immunoblots with synaptosomal, cytosolic, and membrane fractions were probed with an affinity-purified rabbit polyclonal antibody against *hu-syn* (72-10, 1:5000) (Masliah et al., 2000) or with primary antibodies against synaptophysin (1:500, Chemicon, Temecula, CA) or actin (1:1000, Chemicon), followed by secondary goat anti-rabbit or anti-mouse IgG tagged with HRP (1:5000, Santa-Cruz Biotechnology, Inc., Santa Cruz, CA). Blots were visualized by ECL and analyzed with a VersaDoc XL imaging apparatus (BioRad).

#### Neuropathological and Immunocytochemical Analysis

Briefly, as previously described (Masliah et al., 2000), serially sectioned, free-floating, biotin-coded vibratome sections were incubated overnight at 4°C with an anti-*hu-syn*-specific antibody (72-10, 1:5000). To analyze the effects of the immunization in glial cell activation, sections were labeled with mouse monoclonal antibody against the astroglial marker GFAP (1:500, Chemicon) or a rabbit polyclonal antibody against the microglial marker Iba-1 (1:1000, Wako Chemicals, Richmond, VA). Incubation with the primary antibodies was followed by biotinylated goat anti-rabbit IgG or horse anti-mouse IgG (1:100, Vector), Avidin D-HRP (1:200, ABC Elite, Vector), and reaction with DAB tetrahydrochloride containing 0.001%  $H_2O_2$ . Sections were analyzed with the Quantimet 570C (Leica) in order to determine the number of *hu-syn*-immunoreactive inclusions, astroglia, or microglia in the temporal cortex, a brain region often affected in patients with LBD (Hansen et al., 1990). For each case, three sections were analyzed, and the results were averaged and expressed as numbers per square millimeter.

Double-immunocytochemical analysis was performed as previously described (Hashimoto et al., 2004; Hashimoto et al., 2001) to determine the effects of vaccination on nerve terminal density and *hu-syn* accumulation in synapses. Vibratome sections were double-labeled with rabbit polyclonal antibodies against *hu-syn* (72-10, 1:5000) detected with Tyramide Red (1:2000, Roche, Switzerland) and with the mouse monoclonal antibody against synaptophysin (1:15, Chemicon), detected with a horse anti-mouse IgG FITC-tagged secondary antibody (1:75, Vector). For each case, sections were immunolabeled in duplicate and analyzed by LSCM and NIH Image 1.43 software to calculate the percent area of the neuropil covered by synaptophysin-immunoreactive terminals in the temporal cortex (Mucke et al., 2000) and the proportion of synaptophysin-immunoreactive terminals that were *hu-syn* positive (Hashimoto et al., 2004).

Control experiments were performed where sections were immunolabeled with an antibody against *mu-syn* (Masliah et al., 2000), detected with Tyramide Red (1:2000, Roche), or with a rabbit polyclonal antibody against  $\beta$ -syn (1:1000, Chemicon), detected with FITC-tagged goat anti-rabbit secondary antibody (1:75, Vector). In order to confirm the specificity of the primary antibodies, control experiments were performed where sections were incubated overnight in the absence of primary antibody (deleted), with the primary antibody preadsorbed for 48 hr with 20-fold excess of the corresponding peptide or with preimmune serum. To identify if antibodies generated by the vaccinated mice detected *hu-syn* in the tg animals, sections were double labeled with a horse anti-mouse FITC-tagged IgG (1:75, Vector) and a rabbit polyclonal antibody against *hu-syn* (72-10, 1:5000), detected with Tyramide Red (1:2000, Roche). To determine if *hu-syn* colocalized to lysosomes, sections were double labeled with an antibody against *hu-syn* (72-10, 1:5000), detected with Tyramide Red (1:2000, Roche), and a rabbit polyclonal antibody against cathepsin D (1:500, Calbiochem, San Diego, CA), detected with goat anti-rabbit FITC-tagged secondary antibody (1:75, Vector). All sections were processed simultaneously under the same conditions, and experiments were performed twice in order to assess the reproducibility of results. Sections were imaged with a Zeiss 63X (N.A. 1.4) objective on an Axiovert 35 microscope (Zeiss, Germany) with an attached MRC1024 LSCM system (BioRad, Watford, UK) (Masliah et al.,

2000). Images were analyzed with the image program NIH Image 1.43 to determine levels of *mu-syn*,  $\beta$ -syn, or cathepsin D IR.

#### Determination of In Vivo Antibody Recognition of Intracellular *hu-syn*

To further validate whether antibodies against *hu-syn* recognized intracellular *hu-syn*, we generated an FITC-tagged antibody for injection into the brains of nontg and *hu-syn* tg (Line D) mice. For this purpose, a monoclonal antibody that recognizes aa 118–126 of *hu-syn* was generated in mice using recombinant *hu-syn* (clone 9E4, Elan Pharmaceuticals). This antibody was concentrated with a 10 kDa cutoff concentrator centrifuge tube (Millipore, Billerica, MA) and linked to the FITC molecule utilizing a FluoroTag FITC conjugation kit (Sigma) according to the manufacturer's instructions. The FITC-tagged *hu-syn* antibody or a control nonimmune FITC-tagged IgG was injected into the brains of these nontg ( $n = 5$ ) and *hu-syn* tg ( $n = 5$ ) mice (5-month-old). Briefly, as previously described (Marr et al., 2003), mice were placed under anesthesia on a Kopf stereotaxic apparatus, and each mouse was injected with 3  $\mu$ l of FITC-tagged anti-*hu-syn* antibody (approximately 0.5  $\mu$ g/ $\mu$ l) or anti-mouse FITC-tagged IgG into the temporal cortex (using a 5  $\mu$ l Hamilton syringe, 0.25  $\mu$ l/min). After 3 days, mice were anesthetized with chloral hydrate and flush-perfused transcardially with 0.9% saline. Brains were fixed in 4% PFA for 48 hr and vibratomed. Sections from the brains of these mice were then analyzed by direct fluorescence or were commounolabeled with antibodies against *hu-syn* (72-10, 1:1000) or cathepsin D (1:500, Calbiochem), detected with secondary anti-rabbit Texas Red-tagged IgG (1:75, Vector). All sections were imaged with the LSCM as described before.

#### Statistical Analysis

After all results were obtained, the code was broken and statistical comparisons between groups were performed using the two-tailed unpaired Student's *t* test. Linear regression analysis was performed to ascertain the relationship among variables. The Bonferroni correction was applied to account for multiple comparisons.

#### Supplemental Data

The authors' conflict of interest statement and Supplemental Data can be found online at <http://www.neuron.org/cgi/content/full/46/6/857/DC1/>.

#### Acknowledgments

This work was supported by NIH grants AG18440, AG5131, and AG022074 and a grant from Elan Pharmaceuticals. The authors wish to thank Jiping Huang and Robin Barbour (Elan Pharmaceuticals) for preparation of the anti-*hu-syn* synuclein 9E4 antibody.

Received: September 23, 2004

Revised: February 3, 2005

Accepted: May 3, 2005

Published: June 15, 2005

#### References

- Alford, M., Masliah, E., Hansen, L., and Terry, R. (1994). A simple dot-immunobinding assay for the quantification of synaptophysin-like immunoreactivity in human brain. *J. Histochem. Cytochem.* 42, 283–287.
- Bainbridge, J., and Walker, B. (2003). Cell mediated immune responses against human prion protein. *Clin. Exp. Immunol.* 133, 310–317.
- Bard, F., Barbour, R., Cannon, C., Carretto, R., Fox, M., Games, D., Guido, T., Hoenow, K., Hu, K., Johnson-Wood, K., et al. (2003). Epitope and isotype specificities of antibodies to A $\beta$  peptide for protection against AD-like neuropathology. *Proc. Natl. Acad. Sci. USA* 100, 2023–2028.
- Berner, E.J., Mosley, R.L., Destache, C.J., Lewis, T.B., Jackson-Lewis, V., Gorantla, S., Nemerick, C., Green, S.R., Przedborski, S.,

- and Gendelman, H.E. (2004). Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. *Proc. Natl. Acad. Sci. USA* 101, 9435-9440.
- Barbette, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., and Greenamyre, J.T. (2000). Chronic systemic pesticide exposure reproduces features of PD. *Nat. Neurosci.* 3, 1301-1306.
- Braak, H., Del Tredici, K., Braatzke, H., Hamm-Clement, J., Sandmann-Keil, D., and Rub, U. (2002). Staging of the intracerebral inclusion body pathology associated with idiopathic PD (preclinical and clinical stages). *J. Neurol. Suppl.* 3, 1-6.
- Burton, E.A., Giorio, J.C., and Fink, D.J. (2003). Gene therapy progress and prospects. *PD. Gene Ther.* 10, 1721-1727.
- Coleman, T., Ellis, S., Martin, I., Lennard, M., and Tucker, G. (1996). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is N-demethylated by cytochromes P450 2D6, 1A2 and 3A4—implications for susceptibility to PD. *J. Pharmacol. Exp. Ther.* 277, 685-690.
- Cuervo, A.M. (2004). Autophagy: in sickness and in health. *Trends Cell Biol.* 14, 70-77.
- Cuervo, A.M., Stefanis, L., Fredenburg, R., Lansbury, P.T., and Sulzer, D. (2004). Impaired degradation of mutant  $\alpha$ -syn by chaperone-mediated autophagy. *Science* 305, 1292-1296.
- D'Amato, R., Lipman, Z., and Snyder, S. (1986). Selectivity of the parkinsonian neurotoxin MPTP: zinc metabolite MPP<sup>+</sup> binds to neuromelanin. *Science* 231, 987-989.
- Dixon, C., Mathias, N., Zweig, R.M., Davis, D.A., and Gross, D.S. (2005).  $\alpha$ -Syn targets the plasma membrane via the Secretory pathway and induces toxicity in yeast. *Genetics* 170, 47-59. Published on March 21, 2005. 10.1534/genetics.104.035493.
- Dodd, P.R., Hardy, J.A., Oakley, A.E., and Strong, A.J. (1981a). Synaptosomes prepared from fresh human cerebral cortex: morphology, respiration and release of transmitter amino acids. *Brain Res.* 224, 419-425.
- Dodd, P.R., Hardy, J.A., Oakley, A.E., Edwardson, J.A., Perry, E.K., and Delaunoy, J.P. (1981b). A rapid method for preparing synaptosomes: comparison, with alternative procedures. *Brain Res.* 226, 107-118.
- Eliez, D., Kutluay, E., Busell, R., Jr., and Browne, G. (2001). Conformational properties of  $\alpha$ -syn in its free and lipid-associated states. *J. Mol. Biol.* 307, 1061-1073.
- Emadi, S., Liu, R., Yuan, B., Schulz, P., McAllister, C., Lyubchenko, Y., Messer, A., and Sierks, M.R. (2004). Inhibiting aggregation of  $\alpha$ -syn with human single chain antibody fragments. *Biochemistry* 43, 2871-2878.
- Fabian, R.H. (1990). Uptake of antineuronal IgM by CNS neurons: comparison with antineuronal IgG. *Neurology* 40, 419-422.
- Fabian, R.H., and Petroff, G. (1987). Intraneuronal IgG in the central nervous system: uptake by retrograde axonal transport. *Neurology* 37, 1780-1784.
- Feany, M., and Bender, W. (2000). A *Drosophila* model of PD. *Nature* 404, 394-398.
- Ferre, I., Boada Rovira, M., Sanchez Guerra, M.L., Rey, M.J., and Costa-Jussa, F. (2004). Neurotoxicity and pathogenesis of encephalitis following A $\beta$  immunization in AD. *Brain Pathol.* 14, 11-20.
- Forno, L., DeLanney, L., Irwin, I., and Langston, J. (1996). Electron microscopy of Lewy bodies in the amygdala-parahippocampal region. Comparison with inclusion bodies in the MPTP-treated squirrel monkey. In *Advances in Neurology*, L. Battistin, G. Scarlati, T. Caraceni, and S. Ruggieri, eds. (Philadelphia: Lippincott-Raven Publishers), pp. 217-228.
- Frenkel, D., and Solomon, B. (2001). Towards Alzheimer's A $\beta$  vaccination. *Biologicals* 29, 243-247.
- Games, D., Bard, F., Grajeda, H., Guido, T., Khan, K., Sonano, F., Vasquez, N., Wehner, N., Johnson-Wood, K., Yednock, T., et al. (2000). Prevention and reduction of AD-type pathology in PDAPP mice immunized with A $\beta$ 1-42. *Ann. N. Y. Acad. Sci.* 920, 274-284.
- Garzon, J., DeFelipe, J., Rodriguez, J.R., DeAntonio, I., Garcia-Espana, A., and Sanchez-Blazquez, P. (1999). Transport of CSF antibodies to  $\alpha$  subunits across neural membranes requires binding to the target protein and protein kinase C activity. *Brain Res. Mol. Brain Res.* 65, 151-166.
- Gonias, S.L., Wu, L., and Salicioni, A.M. (2004). Low density lipoprotein receptor-related protein: regulation of the plasma membrane proteome. *Thromb. Haemostasis* 91, 1056-1064.
- Hansen, L., and Galasko, D. (1992). Lewy body disease. *Curr. Opin. Neurol. Neurosurg.* 5, 889-894.
- Hansen, L., Salmon, D., Galasko, D., Masliah, E., Katzman, R., DeTeresa, R., Thal, L., Pay, M., Hofstetter, R., and Klauber, M. (1990). The Lewy body variant of AD: a clinical and pathologic entity. *Neurology* 40, 1-7.
- Hashimoto, M., and Masliah, E. (1999).  $\alpha$ -Syn in LBD and AD. *Brain Pathol.* 9, 707-720.
- Hashimoto, M., Hernandez-Ruiz, S., Hsu, L., Sisk, A., Xia, Y., Takeda, A., Sundsmo, M., and Masliah, E. (1998). Human recombinant NACP/ $\alpha$ -syn is aggregated and fibrillated in vitro: Relevance for LBD. *Brain Res.* 799, 301-306.
- Hashimoto, M., Rockenstein, E., Mante, M., Mallory, M., and Masliah, E. (2001).  $\beta$ -Syn inhibits  $\alpha$ -syn aggregation: a possible role as an anti-parkinsonian factor. *Neuron* 32, 213-223.
- Hashimoto, M., Sagara, Y., Everall, I.P., Mallory, M., Everson, A., Langford, D., and Masliah, E. (2002). GFAP regulates signaling via the GSK3 $\beta$  pathway: implications for neuroprotection. *J. Biol. Chem.* 277, 32985-32991.
- Hashimoto, M., Rockenstein, E., Mante, M., Crews, L., Bar-On, P., Gage, F.H., Marr, R., and Masliah, E. (2004). An antiaggregation gene therapy strategy for LBD utilizing  $\beta$ -syn lentivirus in a transgenic model. *Gene Ther.* 11, 1713-1723.
- Herz, J., Kowal, R.C., Ho, Y.K., Brown, M.S., and Goldstein, J.L. (1990). Low density lipoprotein receptor-related protein mediates endocytosis of monoclonal antibodies in cultured cells and rabbit liver. *J. Biol. Chem.* 265, 21355-21362.
- Hock, C., Konietzko, U., Papassotgiropoulos, A., Wolmer, A., Streffer, J., von Rotz, R.C., Davey, G., Moritz, E., and Nitsch, R.M. (2002). Generation of antibodies specific for A $\beta$  by vaccination of patients with Alzheimer disease. *Nat. Med.* 8, 1270-1275.
- Hussain, M.M. (2001). Structural, biochemical and signaling properties of the low-density lipoprotein receptor gene family. *Front. Biosci.* 6, D417-D428.
- Iwai, A. (2000). Properties of NACP/ $\alpha$ -syn and its role in AD. *Biochim. Biophys. Acta* 1502, 95-109.
- Iwai, A., Masliah, E., Yoshimoto, M., De Silva, R., Ge, N., Kittel, A., and Saitoh, T. (1994). The precursor protein of non-A $\beta$  component of AD amyloid (NACP) is a presynaptic protein of the central nervous system. *Neuron* 14, 467-475.
- Iwatsubo, T., Yamaguchi, H., Fujimuro, M., Yokosawa, H., Ihara, Y., Trojanowski, J.O., and Lee, V.-M. (1996). Purification and characterization of Lewy bodies from brains of patients with diffuse LBD. *Am. J. Pathol.* 148, 1517-1529.
- Janus, C., Pearson, J., McLaurin, J., Mathews, P.M., Jiang, Y., Schmidt, S.D., Chishti, M.A., Horne, P., Heslin, D., French, J., et al. (2000). A $\beta$  peptide immunization reduces behavioural impairment and plaques in a model of AD. *Nature* 408, 979-982.
- Jenner, P. (1998). Oxidative mechanisms in nigral cell death in PD. *Mov. Disord.* 13, 24-34.
- Kim, D.W. (2004). Efficient induction of dopaminergic neurons from embryonic stem cells for application to PD. *Yonsei Med. J.* 45 (Suppl.), 23-27.
- Kim, T.D., Pak, S.R., and Yang, C.H. (2002). Structural and functional implications of C-terminal regions of  $\alpha$ -syn. *Biochemistry* 41, 13782-13790.
- Kirk, D., Georgievska, B., and Bjorklund, A. (2004). Localized striatal delivery of GDNF as a treatment for Parkinson disease. *Nat. Neurosci.* 7, 105-110.
- Kosaka, K., Yoshimura, M., Ikeda, K., and Budka, H. (1984). Diffuse type of LBD. Progressive dementia with abundant cortical Lewy bodies and senile changes of varying degree—A new disease? *Clin. Neuropathol.* 3, 183-192.
- Kounnas, M., Moir, R., Rebeck, G., Bush, A., Argaves, W., Tanzi, R.,

- Hyman, B., and Strickland, D. (1995). LDL receptor-related protein, a multifunctional apoE receptor, binds secreted A $\beta$  precursor protein and mediates its degradation. *Cell* 82, 331–340.
- Kruger, R., Kuhn, W., Muller, T., Wotalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J., Schols, L., and Reiss, O. (1998). Ala30Pro mutation in the gene encoding  $\alpha$ -syn in Parkinson's disease. *Nat. Genet.* 18, 108–109.
- Lansbury, P.T.J. (1999). Evolution of amyloid: What normal protein folding may tell us about fibrillogenesis and disease. *Proc. Natl. Acad. Sci. USA* 96, 3342–3344.
- Lee, M.K., Stirling, W., Xu, Y., Xu, X., Qi, D., Mandir, A.S., Dawson, T.M., Copeland, N.G., Jenkins, N.A., and Price, D.L. (2002). Human  $\alpha$ -syn-harboring familial PD-linked Ala53  $\rightarrow$  Thr mutation causes neurodegenerative disease with  $\alpha$ -syn aggregation in transgenic mice. *Proc. Natl. Acad. Sci. USA* 99, 8968–8973.
- Lee, H.J., Khoshaghi, F., Patel, S., and Lee, S.J. (2004a). Clearance of  $\alpha$ -syn oligomeric intermediates via the lysosomal degradation pathway. *J. Neurosci.* 24, 1888–1896.
- Lee, V.M., Giasson, B.I., and Trojanowski, J.Q. (2004b). More than just two peas in a pod: common amyloidogenic properties of tau and  $\alpha$ -syn in neurodegenerative diseases. *Trends Neurosci.* 27, 129–134.
- Lemere, C.A., Spooner, E.T., Leverone, J.F., Mori, C., Iglesias, M., Bloom, J.K., and Seabrook, T.J. (2003). Amyloid- $\beta$  immunization in AD transgenic mouse models and wildtype mice. *Neurochem. Res.* 28, 1017–1027.
- Luthi-Carter, R. (2003). Progress towards a vaccine for Huntington's disease. *Mol. Ther.* 7, 569–570.
- Marr, R.A., Rockenstein, E., Mukherjee, A., Kindy, M.S., Hersh, L.B., Gage, F.H., Verma, I.M., and Masliah, E. (2003). Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J. Neurosci.* 23, 1992–1996.
- Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagar, Y., Sak, A., and Mucke, L. (2000). Dopaminergic loss and inclusion body formation in  $\alpha$ -syn mice: Implications for neurodegenerative disorders. *Science* 287, 1265–1269.
- Masliah, E., Rockenstein, E., Veinbergs, I., Sagar, Y., Mallory, M., Hashimoto, M., and Mucke, L. (2001).  $\beta$ -Amyloid peptides enhance  $\alpha$ -syn accumulation and neuronal deficits in a transgenic mouse model linking AD and PD. *Proc. Natl. Acad. Sci. USA* 98, 12245–12250.
- McKeith, I.G. (2000). Spectrum of PD, Parkinson's dementia, and Lewy body dementia. *Neurol. Clin.* 19, 865–902.
- McLean, P.J., Kawamata, H., Ribich, S., and Hyman, B.T. (2000). Membrane association and protein conformation of  $\alpha$ -syn in intact neurons. Effect of PD-linked mutations. *J. Biol. Chem.* 275, 8812–8816.
- Meeker, M.L., Meeker, R.B., and Hayward, J.N. (1997). Accumulation of circulating endogenous and exogenous immunoglobulins by hypothalamic magnocellular neurons. *Brain Res.* 423, 45–65.
- Miller, T.W., Shriver, T.L., Wolfgang, W.J., Kang, X., and Messer, A. (2003). DNA vaccination against mutant huntingtin ameliorates the HDRE2 diabetic phenotype. *Mol. Ther.* 7, 572–579.
- Morgan, D., Diamond, D., Gottschall, P., Ugen, K., Dickey, C., Hardy, J., Dufré, K., Janzen, P., DiCarlo, G., Wilcock, D., et al. (2000). A $\beta$  peptide vaccination prevents memory loss in an animal model of AD. *Nature* 408, 982–985.
- Mucke, L., Masliah, E., Yu, G.Q., Mallory, M., Rockenstein, E.M., Tsunashima, G., Hu, K., Kholodenko, D., Johnson-Wood, K., and McConlogue, L. (2000). High-level neuronal expression of A $\beta$ 1–42 in wild-type human amyloid precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058.
- Polymeropoulos, M., Lavedan, C., Leroy, E., Ide, S., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., et al. (1997). Mutation in the  $\alpha$ -syn gene identified in families with PD. *Science* 276, 2045–2047.
- Rockenstein, E., Mallory, M., Hashimoto, M., Song, D., Shults, C.W., Lang, I., and Masliah, E. (2002). Differential neuropathological alterations in transgenic mice expressing  $\alpha$ -syn from the platelet-derived growth factor and Thy-1 promoters. *J. Neurosci. Res.* 68, 568–578.
- Salmón, D.P., Galasko, D., Hansen, L.A., Masliah, E., Butters, N., Thal, L.J., and Katzman, R. (1995). Neuropsychological deficits associated with diffuse Lewy body disease. *Brain Cogn.* 37, 148–165.
- Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., et al. (1999). Immunization with A $\beta$  attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400, 173–177.
- Schneider, W.J., and Nimf, J. (2003). LDL receptor relatives at the crossroad of endocytosis and signaling. *Cell. Mol. Life Sci.* 60, 892–903.
- Shastri, B.S. (2001). Parkinson disease: etiology, pathogenesis and future of gene therapy. *Neurosci. Res.* 41, 5–12.
- Sigurdsson, E.M., Brown, D.R., Daniels, M., Kascsak, R.J., Kascsak, R., Carp, R., Meeker, H.C., Frangione, B., and Wisniewski, T. (2002). Immunization delays the onset of prion disease in mice. *Am. J. Pathol.* 161, 13–17.
- Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., et al. (2003).  $\alpha$ -Syn locus triplication causes PD. *Science* 302, 841.
- Spillantini, M., Schmidt, M., Lee, V.-Y., Trojanowski, J., Jakes, R., and Goedert, M. (1997).  $\alpha$ -Syn in Lewy bodies. *Nature* 388, 839–840.
- Takeda, A., Hashimoto, M., Mallory, M., Sundsmo, M., Hansen, L., Sisk, A., and Masliah, E. (1998a). Abnormal distribution of the non-A $\beta$  component of AD amyloid precursor/ $\alpha$ -syn in LBD as revealed by proteinase K and formic acid pretreatment. *Lab. Invest.* 78, 1169–1177.
- Takeda, A., Mallory, M., Sundsmo, M., Honer, W., Hansen, L., and Masliah, E. (1998b). Abnormal accumulation of NACP/ $\alpha$ -syn in neurodegenerative disorders. *Am. J. Pathol.* 152, 367–372.
- Takeda, A., Hashimoto, M., Mallory, M., Sundsmo, M., Hansen, L., and Masliah, E. (2000). C-terminal  $\alpha$ -syn immunoreactivity in structures other than Lewy bodies in neurodegenerative disorders. *Acta Neuropathol. (Berl.)* 99, 296–304.
- Trojanowski, J., and Lee, V. (1998). Aggregation of neurofilament and  $\alpha$ -syn proteins in Lewy bodies: implications for pathogenesis of Parkinson disease and Lewy body dementia. *Arch. Neurol.* 55, 151–152.
- Trojanowski, J., Goedert, M., Iwatsubo, T., and Lee, V. (1998). Fatal attractions: abnormal protein aggregation and neuronal death in PD and Lewy body dementia. *Cell Death Differ.* 5, 832–837.
- Ubol, S., Levine, B., Lee, S.H., Greenspan, N.S., and Griffin, D.E. (1995). Roles of immunoglobulin valency and the heavy-chain constant domain in antibody-mediated downregulation of Sindbis virus replication in persistently infected neurons. *J. Virol.* 69, 1990–1993.
- Veldman, B., Wijn, A., Knoers, N., Praamstra, P., and Horstink, M. (1998). Genetic and environmental risk factors in PD. *Clin. Neurol. Neurosurg.* 100, 15–26.
- Wakabayashi, K., Matsumoto, K., Takayama, K., Yoshimoto, M., and Takahashi, H. (1997). NACP, a presynaptic protein, immunoreactivity in Lewy bodies in PD. *Neurosci. Lett.* 239, 45–48.
- White, A.R., and Hawke, S.H. (2003). Immunotherapy as a therapeutic treatment for neurodegenerative disorders. *J. Neurochem.* 87, 801–808.
- White, A.R., Enever, P., Tayebi, M., Mushens, R., Linehan, J., Brandner, S., Anstee, D., Collinge, J., and Hawke, S. (2003). Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature* 422, 80–83.
- Yoshizaki, T., Inaji, M., Kouike, H., Shimazaki, T., Sawamoto, K., Ando, K., Date, I., Kobayashi, K., Suhara, T., Uchiyama, Y., and Okano, H. (2004). Isolation and transplantation of dopaminergic neurons generated from mouse embryonic stem cells. *Neurosci. Lett.* 363, 33–37.
- Zhou, C., Emadi, S., Sierks, M.R., and Messer, A. (2004). A human single-chain Fv intrabody blocks aberrant cellular effects of over-expressed  $\alpha$ -syn. *Mol. Ther.* 10, 1023–1031.

# Passive Amyloid Immunotherapy Clears Amyloid and Transiently Activates Microglia in a Transgenic Mouse Model of Amyloid Deposition

Donna M. Wilcock,<sup>1,2</sup> Amy N. Rojiani,<sup>1,3,4</sup> Arnon Rosenthal,<sup>5</sup> Gil Levkowitz,<sup>5</sup> Sangeetha Subbarao,<sup>5</sup> Jennifer Alamed,<sup>1,2</sup> David Wilson,<sup>1,2</sup> Nedda Wilson,<sup>1,2</sup> Melissa J. Freeman,<sup>1,2</sup> Marcia N. Gordon,<sup>1,2</sup> and Dave Morgan<sup>1,2</sup>

<sup>1</sup>Alzheimer's Research Laboratory and Departments of <sup>2</sup>Pharmacology, <sup>3</sup>Interdisciplinary Oncology, and <sup>4</sup>Pathology, University of South Florida, Tampa, Florida 33612, and <sup>5</sup>Rinat Neuroscience Corporation, Palo Alto, California 94304

The role of microglia in the removal of amyloid deposits after systemically administered anti- $A\beta$  antibodies remains unclear. In the current study, we injected Tg2576 APP transgenic mice weekly with an anti- $A\beta$  antibody for 1, 2, or 3 months such that all mice were 22 months at the end of the study. In mice immunized for 3 months, we found an improvement in alternation performance in the Y maze. Histologically, we were able to detect mouse IgG bound to congophilic amyloid deposits in those mice treated with the anti- $A\beta$  antibody but not in those treated with a control antibody. We found that Fc $\gamma$  receptor expression on microglia was increased after 1 month of treatment, whereas CD45 was increased after 2 months of treatment. Associated with these microglial changes was a reduction in both diffuse and compact amyloid deposits after 2 months of treatment. Interestingly, the microglia markers were reduced to control levels after 3 months of treatment, whereas amyloid levels remained reduced. Serum  $A\beta$  levels and anti- $A\beta$  antibody levels were elevated to similar levels at all three survival times in mice given anti- $A\beta$  injections rather than control antibody injections. These data show that the antibody is able to enter the brain and bind to the amyloid deposits, likely opsonizing the  $A\beta$  and resulting in Fc $\gamma$  receptor-mediated phagocytosis. Together with our earlier work, our data argue that all proposed mechanisms of anti- $A\beta$  antibody-mediated amyloid removal can be simultaneously active.

**Key words:** Alzheimer's disease; antibody; behavior; microglia; immunization; amyloid  $\beta$

## Introduction

Reduction of brain amyloid after anti- $A\beta$  immunotherapy was first demonstrated by Schenk and colleagues (1999). Their report showed that vaccination with  $A\beta_{1-42}$  in the PDAPP transgenic mouse model of Alzheimer's disease dramatically reduced levels of  $A\beta$  deposits in the brain. Later it was shown that using the same vaccination protocol in APP + PS1 doubly transgenic mice (Morgan et al., 2000) and in TgCRND8 transgenic mice (Janus et al., 2000) not only reduced  $A\beta$  levels in the brain but also protected these mice from memory deficits. More recent studies have demonstrated that passive immunization consisting of direct anti- $A\beta$  antibody injections not only results in dramatic reduction of  $A\beta$  levels (Bard et al., 2000; DeMattos et al., 2001) in the brain but also reverses memory deficits in transgenic mouse models of Alzheimer's disease (Dodart et al., 2002; Kotilinek et al., 2002).

The mechanisms by which immunotherapy acts remain unclear. Suggested mechanisms include microglial-mediated

phagocytosis (Schenk et al., 1999; Wilcock et al., 2001, 2003, 2004), disaggregation of amyloid deposits (Solomon et al., 1997; Wilcock et al., 2003, 2004), and removal of  $A\beta$  from the brain by binding of circulating  $A\beta$  in plasma with the anti- $A\beta$  antibodies, resulting in a concentration gradient from brain to plasma. This latter mechanism is also known as the peripheral sink hypothesis (DeMattos et al., 2001; Dodart et al., 2002; Das et al., 2003; Lemere et al., 2003).

We have previously reported that after intracranial anti- $A\beta$  antibody injections into APP transgenic mice, there is a rapid removal of diffuse amyloid deposits apparently independent of microglial activation and also a later removal of compact amyloid deposits, which appears to require microglial activation (Wilcock et al., 2003). In fact, in a later study using the same model, administration of dexamethasone, which suppresses microglial activation, anti- $A\beta$  antibody administration inhibited the removal of compact, thioflavine-S-positive amyloid deposits (Wilcock et al., 2004).

In this report, we show that weekly systemic administration of anti- $A\beta$  antibodies for 1, 2, or 3 months results in a dramatic reduction of both diffuse and compact amyloid deposits. Associated with this reduction is a behavioral improvement using the Y maze task. After 1 month of treatment, there is a large induction of Fc $\gamma$  receptor expression on microglia, and after 2 months of administration, there is an increase in CD45 expression indica-

Received March 24, 2004; revised May 12, 2004; accepted May 13, 2004.

This work was supported by National Institutes on Aging Grants AG-5490 (M.N.G.) and AG-18478 (D.M.) from the National Institutes of Health, D.M.W. is a Benjamin Franklin Fellow in Alzheimer's disease research.

Correspondence should be addressed to Dr. Dave Morgan, Department of Pharmacology, University of South Florida, 12901 Bruce B. Downs Boulevard, MDC Box 9, Tampa, FL 33612. E-mail: dmorgan@usf.edu.

DOI: 10.1523/JNEUROSCI.1090-04.2004

Copyright © 2004 Society for Neuroscience 0270-6474/04/246144-08\$15.00/0

tive of microglial activation. We have detected antibody binding to congophilic plaque in APP transgenic mice treated with an anti- $A\beta$  antibody. We also observed a dramatic increase in circulating  $A\beta$  levels after 1 month of administration. Two months after administration, we observed a dramatic reduction in compact and diffuse deposits. After 3 months of administration, the microglia markers are down to control levels, whereas the compact and diffuse amyloid deposits remain reduced. These results demonstrate systemically administered anti- $A\beta$  antibodies are accessing the brain, binding to amyloid deposits, and activating microglia. The data also show an increase in circulating  $A\beta$  in plasma, consistent with the peripheral sink hypothesis.

## Materials and Methods

**Experiment design.** Singly transgenic APP Tg2576 mice were obtained from our breeding program at University of South Florida, started in 1996 (Hukuhara et al., 1998). Twenty-two APP transgenic mice aged 19 months were assigned to one of four experimental groups. The first three groups received weekly intraperitoneal anti- $A\beta$  antibody injections (antibody 2286, mouse monoclonal anti-human  $A\beta_{1-40}$  IgG1; Rinat Neuroscience Corporation) for 1 month ( $n = 6$ ), 2 months ( $n = 9$ ), or 3 months ( $n = 4$ ). The fourth group received weekly intraperitoneal anti-AMN antibody injections (2906; mouse monoclonal anti-Drosophila amnesiac protein IgG1; Rinat Neuroscience Corporation) for 3 months ( $n = 3$ ). Twelve nontransgenic mice were assigned to one of two experimental groups. The first group received intraperitoneal anti- $A\beta$  antibody injections for 3 months ( $n = 4$ ). The second group received no treatment ( $n = 3$ ). All mice were given a dose of 10 mg/kg of the appropriate antibody. Treatment of 1 and 2 month groups was delayed to insure the mice were killed at the same age (22 months). One week before killing and 1 d after the 5th, 9th, or 13th injection, mice were tested behaviorally using the Y maze task.

**Behavioral testing.** The Y maze is a three-arm maze with equal angles between all arms. Mice were initially placed within one arm, and the sequence and number of arm entries were recorded for each mouse over an 8 min period. The percentage of trials in which all three arms were represented (ABC, CAB, or BCA but not BAB) was recorded as an indicator to estimate short-term memory of the last arms entered. The total number of possible alternations is the number of arm entries minus two. Additionally, the number of arm entries serves as an indicator of activity.

**Antibody purification.** Antibody 2286 (mouse monoclonal anti-human  $A\beta_{1-40}$  IgG1) and antibody 2906 (mouse monoclonal anti-Drosophila amnesiac protein IgG1) were purified from mouse ascites on AKTA instrumentation using protein A beads (MabSelect; Amersham Biosciences, Arlington Heights, IL). Briefly, ascites was filtered in a pyrogen-free 0.22  $\mu$ m filter system (Corning, Corning, NY) and applied to a 20 ml bed volume in an XK16/20 column (Amersham Biosciences), after equilibrating the beads with 5 vol of binding buffer (0.6 M NaCl and 0.3 M glycine, pH 8.0). The column was washed with 3 vol of binding buffer, and the antibody was eluted in 4 vol of elution buffer (0.1 M Na citrate, pH 3.0) and held at low pH for 30 min for viral inactivation. The resulting eluant was neutralized with 0.1 vol of 1.0 M Tris, pH 9.5. The antibody was dialyzed into sterile PBS, pH 7.4, and the concentration was determined by reading absorbance at 280 nm. All buffers were made in pyrogen-free water.

**Tissue preparation.** On the day of killing, mice were weighed and overdosed with 100 mg/kg pentobarbital (Nembutal sodium solution; Abbott Laboratories, North Chicago, IL). Blood was collected and allowed to coagulate at 4°C for at least 1 hr before being centrifuged, and the serum was removed and stored at  $-80^{\circ}\text{C}$  until required. The mice were then intracardially perfused with 25 ml of 0.9% sodium chloride. Brains were rapidly removed, and the right half of the brain was dissected and frozen for biochemistry, whereas the left half of the brain was immersion-fixed for 24 hr in freshly prepared 4% paraformaldehyde in 100 mM  $\text{PO}_4$ , pH 7.2, for histopathology. The latter hemibrains were then incubated for 24 hr in 10, 20, and 30% sucrose sequentially to cryoprotect them. Horizontal sections of 25  $\mu$ m thickness were collected using a sliding microtome

and stored at 4°C in Dulbecco's PBS with sodium azide, pH 7.2, to prevent microbial growth.

**ELISA methods:  $A\beta$  and anti- $A\beta$  antibody.** For the  $A\beta$  assay, serum was diluted and incubated in 96-well microtiter plates (MaxiSorp; Nunc, Roskilde, Denmark), which were precoated with antibody 6E10 (Signt, Dedham, MA) at 5  $\mu$ g/ml in PBS buffer, pH 7.4. The secondary antibody was biotinylated 4C8 (Signt) at a 1:5000 dilution. Detection was done using a streptavidin-horseradish peroxidase conjugate (Amersham Biosciences), followed by TMB substrate (KPL, Gaithersburg, MD). Standard curves of  $A\beta_{1-40}$  (Global Peptide, Ft. Collins, CO) scaling from 6–400 pm were used.

The anti- $A\beta$  antibody was dissociated from endogenous  $A\beta$  in serum as described previously (Li et al., 2004). Briefly, serum was diluted in dissociation buffer (0.2 M glycine HCl and 1.5% BSA, pH 2.5) and incubated at room temperature for 20 min. The sera were pipetted into the sample reservoir of a Microcon centrifugal device (10,000 molecular weight cutoff; YM-10; Millipore, Bedford, MA) and centrifuged at 8000  $\times$  g for 20 min, at room temperature. The sample reservoir was then separated from the flow-through, placed inverted into a second tube, and centrifuged at 1000  $\times$  g for 3 min. The collected solution containing the antibody dissociated from the  $A\beta$  peptide was neutralized to pH 7.0 with 1 M Tris buffer, pH 9.5. The dissociated sera were assayed by ELISA for antibody titer.  $A\beta_{1-40}$  (Global Peptide)-coated 96-well microtiter plates (MaxiSorp; Nunc) were incubated with dissociated serum samples. A biotinylated goat anti-mouse IgG (heavy and light chain; Vector Laboratories, Burlingame, CA) at 1:5000 dilution followed by peroxidase-conjugated streptavidin (Amersham Biosciences) was used to detect serum anti- $A\beta$  binding activity.

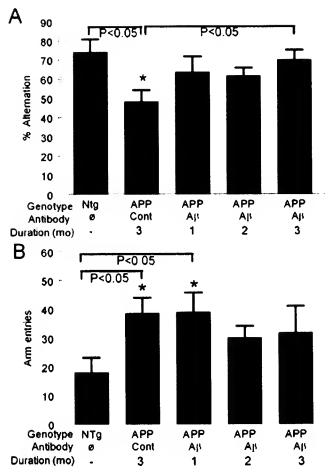
**Immunohistochemical methods.** A series of eight equally spaced tissue sections 2.4 mm apart were randomly selected, spanning the entire brain, and stained using free-floating immunohistochemistry methods for total  $A\beta$  (rabbit polyclonal anti-pan- $A\beta$ ; 1:10,000; Bioscience Resource, Camarillo, CA), CD45 (rat anti-mouse CD45; 1:3000; Serotec, Raleigh, NC), and Fc $\gamma$  receptors II and III (rat anti-mouse CD16 and CD32; 1:3000; BDB Pharmingen, San Diego, CA) as previously described (Gordon et al., 2002). Briefly, tissue was incubated in primary antibody overnight at room temperature. Sections were then washed and incubated in the appropriate biotinylated secondary antibody (for  $A\beta$ , goat anti-rabbit, 1:3000; for CD45 and Fc $\gamma$ , goat anti-rat, 1:1000; all Vector Laboratories) for 2 hr. After multiple washes, tissue was incubated in ABC (Vector Laboratories) for 1 hr. Color development was performed using 3,3'-diaminobenzidine (DAB; Sigma, St. Louis, MO) enhanced with nickelous ammonium sulfate (J. T. Baker Chemical Company, Phillipsburg, NJ) for CD45 and Fc $\gamma$  or without enhancement for  $A\beta$ . For immunostaining, some sections were omitted from the primary antibody to assess nonspecific immunohistochemical reactions.

Additional sections were also stained for mouse IgG using immunohistochemical methods similar to that described above. Briefly, sections were incubated overnight in 1:3000 concentration of anti-mouse IgG conjugated to horseradish peroxidase (Sigma). The sections were then washed and incubated for 5 min in 100 ml of Tris-buffered saline containing 50 mg of DAB (Sigma), 500 mg of nickelous ammonium sulfate (J. T. Baker Chemical Company), and 100  $\mu$ l of 30% hydrogen peroxide to produce a purple-black color reaction product. The sections were then mounted on slides and counterstained with a 0.2% Congo red solution in 80% ethanol to assess the localization of positive mouse IgG stain with compact amyloid deposits.

A set of sections was also mounted and stained using 0.2% Congo red solution in NaCl-saturated 80% ethanol. Another set of sections was also mounted and stained using 4% thioflavin-S (Sigma) for 10 min.

The immunohistochemical reaction product on all sections was measured using Image-Pro Plus version 4.5 software (Media Cybernetics, Silver Spring, MD). One region of the frontal cortex for all sections from each animal was analyzed, and an average of six or seven sections was taken to give a value for each animal. Three regions of the hippocampus were analyzed on approximately four or five sections where hippocampus was present: cornu ammonis 1 (CA1), CA3, and dentate gyrus. These regions were analyzed both individually to yield an average per region and also combined to give an overall value for the hippocampus for each





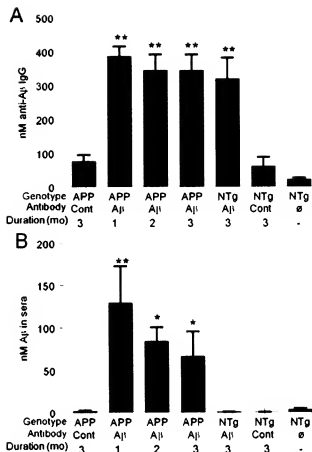
**Figure 1.** Y maze behavioral improvement after systemic anti-A $\beta$  antibody administration. *A*, Percent alternation for nontransgenic (NTg) mice receiving no treatment ( $\emptyset$ ), APP transgenic mice (APP) receiving the control antibody (Cont) for 3 months, and APP transgenic mice (APP) receiving the anti-A $\beta$  (A $\beta$ ) antibody for 1, 2, or 3 months. \* $p < 0.05$  when compared with nontransgenic untreated mice and APP transgenic mice receiving the anti-A $\beta$  antibody for 3 months. *B*, Number of arm entries for nontransgenic mice receiving no treatment, APP transgenic mice receiving the control antibody for 3 months, and APP transgenic mice receiving the anti-A $\beta$  antibody for 1, 2, or 3 months. \* $p < 0.05$  when compared with nontransgenic untreated mice.

animal. This ensured that there was no regional bias in the hippocampal values. These same analysis methods were also used to evaluate the Congo red stain. To assess possible treatment-related differences, the values for each treatment group were analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD) means comparisons. Nontransgenic mice showed no treatment-related differences in any histological analyses, so these groups were pooled.

**Data analysis.** Percent alternation and arm entry numbers from the Y maze behavior task were analyzed using one-way ANOVA followed by Fisher's LSD means comparisons using StatView software version 5.0.1 (SAS Institute Inc., Cary, NC). Nontransgenic mice showed no treatment-related differences in any behavioral analyses, so these groups were pooled. ELISA values for serum A $\beta$  levels and circulating antibody levels were analyzed using one-way ANOVA followed by Fisher's LSD means.

## Results

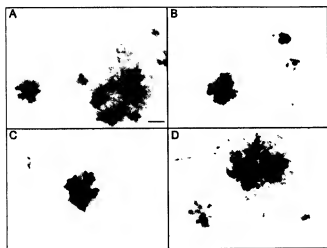
Transgenic APP mice given control antibody injections showed significantly reduced Y maze alternation when compared with the nontransgenic mice (Fig. 1*A*). This reduced alternation was reversed in the APP transgenic mice receiving weekly anti-A $\beta$  antibody injections for 3 months. This group of mice was indistinguishable from the nontransgenic animals and showed signif-



**Figure 2.** Increased serum levels of anti-A $\beta$  antibody and A $\beta$  after anti-A $\beta$  antibody administration. *A*, Amounts of circulating anti-A $\beta$  antibodies in APP transgenic mice (APP) receiving either the control antibody (Cont) for 3 months or the anti-A $\beta$  antibody (A $\beta$ ) for 1, 2, or 3 months, nontransgenic (NTg) mice receiving either the control antibody or the anti-A $\beta$  antibody for 3 months, and nontransgenic mice receiving no treatment. \*\* $p < 0.001$  compared with APP mice given control antibody injections. *B*, Amounts of circulating A $\beta$  in sera in APP transgenic mice receiving either the control antibody for 3 months or the anti-A $\beta$  antibody for 1, 2, or 3 months, nontransgenic mice receiving either the control antibody (Cont) or the anti-A $\beta$  antibody for 3 months, and nontransgenic mice receiving no treatment. \*\* $p < 0.01$ ; \* $p < 0.05$ .

icantly increased alternation compared with the APP transgenic mice receiving the control antibody (Fig. 1*A*). The APP transgenic mice given weekly anti-A $\beta$  antibody injections for either 1 or 2 months were intermediate between nontransgenic and transgenic mice given control antibodies and not significantly different from either group. Nontransgenic mice also made significantly fewer arm entries than the APP transgenic mice receiving control antibody injections, indicating hyperactivity in the APP transgenic mice. The APP transgenic mice receiving anti-A $\beta$  antibody injections for 2 and 3 months did not exhibit this hyperactivity and were not significantly different from any other treatment groups (Fig. 1*B*).

One day after anti-A $\beta$  antibody administration, anti-A $\beta$  antibodies were detected in serum at high levels (400 nM) after 1 month of administration. This level of antibody in the serum was the same after 2 or 3 months of administration, with no apparent accumulation of the antibody (Fig. 2*A*). Associated with high anti-A $\beta$  antibody levels in serum at 1 month was a dramatic increase in circulating A $\beta$  levels in serum. APP transgenic mice receiving the control antibody had only 1.5 nM circulating A $\beta$  in



**Figure 3.** Mouse IgG immunohistochemistry showing antibody binding to Congoophilic plaques in anti- $A\beta$  antibody-treated mice but not control antibody-treated mice. *A–D*, Anti-mouse IgG-HRP immunohistochemistry counterstained with Congo red to detect compact amyloid deposits. *A*, Representative amyloid deposit and associated anti-mouse IgG immunostaining (black) in the hippocampus of a mouse injected with the control antibody for 3 months. *B–D*, Representative amyloid deposit (red) associated with anti-mouse IgG immunostaining (black) in the hippocampus of a mouse injected with the anti- $A\beta$  antibody for 1 month (*B*), 2 months (*C*), or 3 months (*D*). Magnification, 200 $\times$ . Scale bar, 25  $\mu$ m.

plasma compared with APP transgenic mice receiving the  $A\beta$  antibody for 1 month, which had 130 nM circulating  $A\beta$  in plasma, an almost 100-fold increase (Fig. 2*B*). Despite similar levels of anti- $A\beta$  antibody at 1, 2, or 3 months of administration, circulating  $A\beta$  levels declined between 1 and 2 months. They also showed a slight decline between 2 and 3 months of administration, although with both 2 and 3 months of administration, circulating  $A\beta$  levels were still significantly elevated compared with APP transgenic mice receiving the control antibody (Fig. 2*B*).

After systemic administration of anti- $A\beta$  antibodies weekly for 1 month, staining for mouse IgG could be detected on plaques throughout the brains of APP transgenic mice (Fig. 3*B*). The staining was the most intense where plaque load was greatest: the hippocampus and frontal cortex. This staining was not observed in APP transgenic mice receiving the control antibody (Fig. 3*A*). It should be noted that staining with higher concentrations of anti-mouse IgG-HRP did show staining of plaques in both control-treated and anti- $A\beta$  treated APP transgenic mice. Staining for mouse IgG was still present and slightly more intense around the plaques that remained after 2 (Fig. 3*C*) and 3 (Fig. 3*D*) months of treatment.

Total  $A\beta$  immunohistochemistry in the APP transgenic mice receiving control antibody (Fig. 4*A*) showed a few intensely stained deposits, suggesting compacted amyloid deposits along with more numerous diffuse deposits. There was a concentration of deposits around the hilus of the hippocampus as well as the molecular layers of Ammon's horn. This was a typical amount and distribution of  $A\beta$  for APP transgenic mice of this age, as previously described (Hsiao et al., 1996; Gordon et al., 2002). After 1 month of weekly anti- $A\beta$  antibody injections, there appeared to be a slight reduction in  $A\beta$  immunohistochemistry in the hippocampus (Fig. 4*B*), although this was not statistically significant (Fig. 4*E*). The reduction appeared to be primarily diffuse deposits, with most of the compact amyloid deposits remaining (Fig. 4*B*). After 2 months of weekly anti- $A\beta$  antibody injections, we observed a dramatic reduction in  $A\beta$  immunohis-

tochemistry, which appeared to be both compact and diffuse amyloid deposits from the hilus and dentate gyrus regions of the hippocampus as well as the pyramidal cell regions, with only a few deposits remaining, often in the vicinity of the hippocampal fissure and outer molecular layers (Fig. 4*C*). This reduction in  $A\beta$  load at 2 months was  $\sim 60\%$  in the hippocampus and  $\sim 55\%$  in the frontal cortex (Fig. 4*E*) (hippocampus,  $p < 0.001$ ; frontal cortex,  $p < 0.005$ ). Total  $A\beta$  levels remained reduced after 3 months of treatment but did not appear to decrease any further (Fig. 4*D,E*).

Congo red staining detects only compact amyloid deposits in the  $\beta$  pleated sheet structure. There were far fewer Congo red-positive amyloid deposits than  $A\beta$  deposits detected by total  $A\beta$  immunohistochemistry. Congo red-positive deposits were located primarily along the fissure of the hippocampus as well as the CA1/subiculum region in APP transgenic mice receiving the control antibody (Fig. 5*A*). There was no reduction in Congoophilic deposits 1 month after treatment in either the hippocampus (Fig. 5*B,E*) or the frontal cortex (Fig. 5*E*). After 2 months of treatment, there was a significant reduction in both the number and size of Congoophilic deposits in both the hippocampus (Fig. 5*C,E*) and frontal cortex (Fig. 5*E*). This reduction was  $\sim 60\%$  in the frontal cortex and  $\sim 50\%$  in the hippocampus (Fig. 5*E*) (frontal cortex,  $p < 0.005$ ; hippocampus,  $p < 0.01$ ). There was a small further reduction between 2 and 3 months, which was  $\sim 30\%$  in the hippocampus and frontal cortex (Fig. 5*E*). Thioflavine-S staining was also measured and confirmed the Congo red data, showing the same reductions in the stained area as did Congo red (data not shown).

Immunohistochemical staining for Fc $\gamma$  receptors II and III in APP transgenic mice receiving control antibody treatment for 3 months showed only very faint staining of microglia in close association with amyloid deposits (Fig. 6*A*). After 1 month of anti- $A\beta$  antibody administration, there was a dramatic induction of Fc $\gamma$  receptors II and III on microglia. The microglia expressing the Fc $\gamma$  receptors after 1 month of treatment were not only associated with amyloid deposits but also diffusely distributed (Fig. 6*B*). This induction averaged 100-fold in the hippocampus (Fig. 6*B,E*) ( $p < 0.05$ ) and frontal cortex (Fig. 6*E*) ( $p < 0.05$ ). Fc $\gamma$  receptor expression levels fell only slightly between 1 and 2 months of treatment, although this expression was once again concentrated on microglia around remaining amyloid deposits (Fig. 6*D*). Induction remained  $\sim 100$ -fold in the hippocampus (Fig. 6*E*) ( $p < 0.05$ ) and frontal cortex (Fig. 6*E*) ( $p < 0.05$ ). After 3 months of treatment, Fc $\gamma$  receptor expression was reduced to levels observed in APP transgenic mice receiving the control antibody (Fig. 6*E*), although it appeared to be increased in microglia around the few remaining amyloid deposits (Fig. 6*D*).

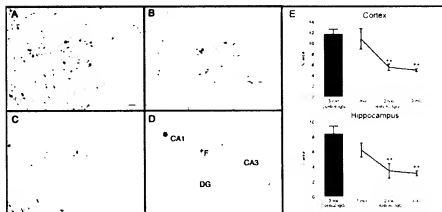
CD45, a protein-tyrosine phosphatase, is normally moderately expressed on microglia around amyloid deposits in aged APP transgenic mice and is a commonly used marker for microglial activation. This moderate expression was observed in the APP transgenic mice receiving control antibody treatment for 3 months (Fig. 7*A,E*). After 1 month of treatment, we observed an increase in CD45 expression on microglia surrounding amyloid deposits in both the hippocampus (Fig. 7*B,F,I*) and frontal cortex (Fig. 7*I*). Although expression in the hippocampus was  $\sim 2.5$  times that observed in control-treated APP transgenic mice (Fig. 7*I*) (not significant) and twice the values found in the frontal cortex of control animals, the elevation was not statistically significant (Fig. 7*I*). After 2 months of anti- $A\beta$  antibody treatment, there was an additional increase in CD45 expression on microglia not only surrounding the amyloid deposits but also diffusely dis-

tributed throughout the amyloid-containing brain regions (Fig. 7C,G). It is possible that this more widespread activation is in association with diffuse amyloid deposits, although we cannot confirm this. The increased expression was  $\sim 3.5$  times that observed in control-treated mice in the hippocampus (Fig. 7I) ( $p < 0.05$ ) and 3 times in the frontal cortex (Fig. 7I) ( $p < 0.01$ ). After 3 months of anti- $A\beta$  antibody treatment, CD45 expression remained at the same levels as that observed after 1 month of treatment (Fig. 7I); however, the microglia were still diffusely distributed, with fewer microglia around deposits compared with 1 or 2 months of treatment (Fig. 7D,H).

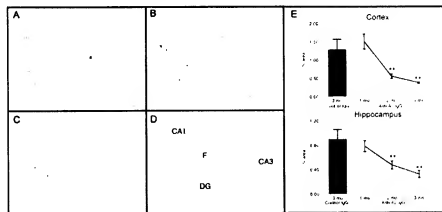
## Discussion

The data presented here suggest that peripherally administered anti- $A\beta$  antibodies entered the brain, bound to congophilic amyloid plaques, and led to removal of deposited amyloid. In support of the argument that anti- $A\beta$  antibodies entered the brain, we found mouse IgG marking the remaining congophilic amyloid plaques of APP transgenic mice administered the anti- $A\beta$  antibody but no IgG in APP transgenic mice administered the control antibody. This difference was best discerned when low titers of the anti-mouse IgG-HRP were used. Seabrook et al. (2004) also reported immunohistochemical labeling of amyloid deposits for mouse IgG after passive immunization but detected signals in both immunized and nonimmunized mice. It is unclear whether lower anti-mouse IgG concentrations might have revealed selective staining in anti- $A\beta$ -treated animals. These data confirm in paraffinaldehyde-fixed tissue the observations of Bard et al. (2000), who used unfixed cryostat sections.

Associated with the presence of the antibody in the brain after 1 month of treatment was a dramatic activation of Fc $\gamma$  receptor expression on microglia, further arguing that anti- $A\beta$  antibodies entered the brain and opsonized the amyloid deposits. Later, after 2 months of treatment, we observed an increase in CD45 expression on microglia, indicating activation of these cells beyond the level normally associated with amyloid deposits. It has previously been shown that after active immunization with  $A\beta_{1-42}$  in humans, anti- $A\beta$  antibodies are present in CSF, in some instances equal to the serum concentration, suggesting some penetration into the brain from the periphery (Hock et al., 2002). It has also been shown that 0.1% of an intravenous injection of a radiolabeled anti- $A\beta$  antibody crosses the blood–brain barrier of SAMP8 mice (Banks et al., 2002). Thus, accumulating data indicate that circulating antibodies can access the brain parenchyma, which has important implications not only for the use of immunotherapy in Alzheimer's disease but also for other dis-



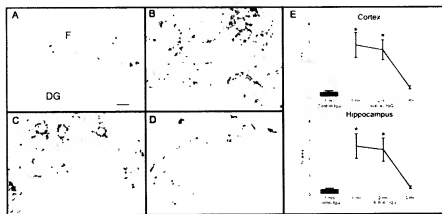
**Figure 4.** Total  $A\beta$  immunohistochemistry is reduced after 2 months of systemic anti- $A\beta$  antibody administration. *A–D*, Total  $A\beta$  immunohistochemistry in the hippocampus of APP transgenic mice receiving the control antibody for 3 months (*A*; percent area for this section was 9.12%), the anti- $A\beta$  antibody for 1 month (*B*; percent area for this section was 6.84%), the anti- $A\beta$  antibody for 2 months (*C*; percent area for this section was 3.23%), or the anti- $A\beta$  antibody for 3 months (*D*; percent area for this section was 2.49%). Magnification,  $40\times$ . Scale bar,  $120\ \mu\text{m}$ . *E*, Quantification of the percent area occupied by the  $A\beta$ -positive stain in the frontal cortex and hippocampus. The single bar shows the value for APP transgenic mice receiving the control antibody for 3 months. The line shows the values for APP transgenic mice receiving the anti- $A\beta$  antibody for 1, 2, and 3 months.  $^{**}p < 0.01$ . CA1, Cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus; F, hippocampal figure.



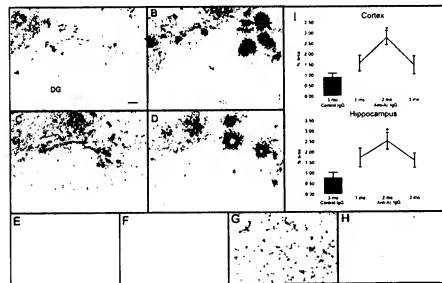
**Figure 5.** Congophilic compact amyloid plaques are reduced after 2 months of anti- $A\beta$  antibody administration. *A–D*, Congo red staining in the hippocampus of APP transgenic mice receiving the control antibody for 3 months (*A*), the anti- $A\beta$  antibody for 1 month (*B*), the anti- $A\beta$  antibody for 2 months (*C*), or the anti- $A\beta$  antibody for 3 months (*D*). Magnification,  $40\times$ . Scale bar,  $120\ \mu\text{m}$ . *E*, Quantification of the percent area occupied by the Congo red-positive stain in the frontal cortex and hippocampus. The single bar shows the value for APP transgenic mice receiving the control antibody for 3 months. The line shows the values for APP transgenic mice receiving the anti- $A\beta$  antibody for 1, 2, and 3 months.  $^{**}p < 0.01$ . CA1, Cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus; F, hippocampal figure.

eases in which immunotherapy is being pursued, such as Creutzfeldt–Jakob disease (Manuelidis, 1998; Sigurdsson et al., 2003) and neural infections associated with human immunodeficiency virus (McMichael and Hanke, 2003).

Associated with the changes in microglial markers was a significant reduction in both compact and diffuse amyloid deposits after 2 months of treatment; these remained reduced after 3 months of treatment. Removal of  $A\beta$  deposits from the brain appeared to be a gradual process. We did not observe significant reductions in either diffuse or compact amyloid deposits after 1 month of weekly anti- $A\beta$  antibody treatment. After 2 months of treatment, there was a dramatic reduction in total  $A\beta$  immunohistochemistry, Congo red staining, and thioflavine-S staining, suggesting removal of both diffuse and compact amyloid deposits. There appeared to be no accumulation of the injected antibody because serum anti- $A\beta$  antibody levels were the same re-



**Figure 6.** Fc $\gamma$  receptor expression on microglia is increased after 1 month of anti-A $\beta$  antibody treatment and remains increased after 2 months of treatment. *A–D*, Fc $\gamma$  receptor immunohistochemistry in the hippocampus of APP transgenic mice receiving the control antibody for 3 months (*A*), the anti-A $\beta$  antibody for 1 month (*B*), the anti-A $\beta$  antibody for 2 months (*C*), or the anti-A $\beta$  antibody for 3 months (*D*). Magnification, 100 $\times$ . Scale bar, 50  $\mu$ m. *E*, Quantification of the percent area occupied by the Fc $\gamma$  receptor-positive stain in the frontal cortex and hippocampus. The single bar shows the value for APP transgenic mice receiving the control antibody for 3 months. The line shows the values for APP transgenic mice receiving the anti-A $\beta$  antibody for 1, 2, and 3 months. \* $p < 0.05$ . DG, Dentate gyrus; F, hippocampal fissure.



**Figure 7.** CD45 expression on microglia is increased after 2 months of anti-A $\beta$  antibody treatment. *A–D*, CD45 immunohistochemistry in the hippocampus of APP transgenic mice receiving the control antibody for 3 months (*A*), the anti-A $\beta$  antibody for 1 month (*B*), the anti-A $\beta$  antibody for 2 months (*C*), or the anti-A $\beta$  antibody for 3 months (*D*). *E*, Hippocampal fissure; DG, dentate gyrus. Magnification, 100 $\times$ . Scale bar, 50  $\mu$ m. *E–H*, Magnified images of non-amyloid-containing areas from *A–D*, showing CD45 immunohistochemistry in the hippocampus of APP transgenic mice receiving the control antibody for 3 months (*E*), the anti-A $\beta$  antibody for 1 month (*F*), the anti-A $\beta$  antibody for 2 months (*G*), or the anti-A $\beta$  antibody for 3 months (*H*). *I*, Quantification of the percent area occupied by the CD45-positive stain in the frontal cortex and hippocampus. The single bar shows the value for APP transgenic mice receiving the control antibody for 3 months. The line shows the values for APP transgenic mice receiving the anti-A $\beta$  antibody for 1, 2, and 3 months. \* $p < 0.05$ .

regardless of the duration of treatment. This would suggest that this time-dependent removal of amyloid deposits was not occurring because of increasing antibody levels; rather, it appears that some mobilization of removal mechanisms must be present for some time before significant removal is apparent.

An early feature we observed was the increase in Fc $\gamma$  receptor II and III (CD16 and CD32) expression on microglia, which was apparent after 1 and 2 months of treatment. The murine Fc $\gamma$  receptors II and III share a high affinity for IgG1 antibodies (the isotype used in the current study) as well as IgG2a (Gessner et al.,

1998). Following this increased Fc $\gamma$  receptor expression was an increase in CD45 expression on microglia after 2 months of treatment. CD45 is a protein-tyrosine phosphatase, which is elevated with microglial activation. In this study, it appears that the increase in CD45 expression represents a further activation step from that seen after 1 month of treatment, at which we observed the increased Fc $\gamma$  receptor expression. After 3 months of treatment, both Fc $\gamma$  receptor and CD45 expression on microglia were reduced to control levels, possibly because of the substantial reduction in amyloid deposits. It is important to note that if we had looked at only the 3 month point, we would not have detected the activation of the microglia by CD45 or, likely, by other markers such as Mac-1 (Das et al., 2003).

We have previously observed a similar loss of microglia activation after intracranial antibody administration (Wilcock et al., 2003) and active immunization (Wilcock et al., 2001). Three days after a single injection of anti-A $\beta$  antibody in the frontal cortex and hippocampus, we observed an increase in CD45 expression; however, 7 d after injection, the CD45 expression was reduced to control levels, in parallel with clearance of the A $\beta$  deposits (Wilcock et al., 2003). This suggests that the reduced microglial activation could possibly be attributable to the clearance of most amyloid plaques. It is also conceivable that the microglia could be undergoing apoptosis attributable to the robust activation, as has been described previously by Liu et al. (2001), when microglia are overactivated by lipopolysaccharide. An alternative explanation could be tolerance of the microglia to antibody-opsonized A $\beta$ . We have previously shown a reduction in microglial reaction in an active immunization model using doubly transgenic APP + PS1 mice. After five monthly inoculations, we observed a significant increase in CD45 expression; however, after nine monthly inoculations, CD45 levels were comparable with those of control animals despite continued high antibody titer levels (Wilcock et al., 2001).

Because of the inflammatory adverse effects seen in a human clinical trial of the active immunization by Elan Pharmaceuticals (Ferrer et al., 2004), it could be suggested that the microglial activation observed in this study was attributable to an immune response unrelated to opsonization of A $\beta$  by the antibody. To evaluate whether this was the case, we examined the thalamus and cerebellum, which do not contain any amyloid deposits, for any increase in CD45 or Fc $\gamma$  receptor expression and did not observe any such increase. Thus, it appears that the microglial activation is specific to amyloid-containing brain regions and is likely a specific response to opso-

nized A $\beta$  as opposed to a general nonspecific inflammatory reaction.

The data presented here extend our previous observations of the benefits of active anti-A $\beta$  immunization on learning and memory (Morgan et al., 2000). We show that passive immunization with anti-A $\beta$  antibodies for 3 months reduced amyloid deposits and improved behavioral performance, as indicated by a significant increase in alternation in the Y maze as well as a decrease in the number of arm entries. The arm entry data suggest that there is not a complete reversal of the increased activity. There is a trend toward some improvement in alternation at the 1 month point (although not significant) despite no reduction in total A $\beta$  immunohistochemistry. Such improvements may reflect rapid reductions of an A $\beta$  pool (oligomeric?) closely linked to memory impairments yet not easily detected by immunohistochemistry. This phenomenon was previously described by Dodart et al. (2002) and Kotilinek et al. (2002), who showed rapid reversal of memory deficits in transgenic mice after passive immunization without significant reduction in brain A $\beta$ .

The results described above indicating entry of the anti-A $\beta$  antibody into the brain and activation of microglia suggest that some opsonization of A $\beta$  is likely stimulating microglial involvement in the clearance of A $\beta$  deposits. This is consistent with the phagocytosis mechanisms of amyloid removal put forward by the Llan Pharmacological group (Schenk et al., 1999; Bard et al., 2000, 2003). Our earlier work with direct injection of the anti-A $\beta$  antibody into the brain suggests two mechanisms: one not requiring an Fc component or activation of microglia, which can clear diffuse A $\beta$ , and a second that requires the Fc domain and activation of microglia (Wilcock et al., 2003, 2004). It is conceivable that the first non-Fc-requiring mechanism is analogous to the catalytic dissolution mechanism described by Solomon et al. (1997). The diffuse material, whatever its state of oligomerization, may be more accessible to this action of anti-A $\beta$  antibodies. Finally, at all durations of antibody exposure, we observed a dramatic increase in circulating A $\beta$  levels in plasma. This is consistent with a role for the peripheral sink mechanism (DeMattos et al., 2001; Dodart et al., 2002; Lemere et al., 2003) in the reduction of CNS A $\beta$  after passive immunization.

We conclude that our studies using antibody 2286, in aggregate, provide support for all three major proposed mechanisms of anti-A $\beta$  antibody action in lowering brain amyloid. It is essential to recognize that these mechanisms are not mutually exclusive and are likely to be synergistic if multiple mechanisms are elicited by a single antibody or serum. It is also important to recognize that not all monoclonal antibodies need work via all three mechanisms. Both isotype and epitope selectivity could regulate which anti-A $\beta$  action is dominant for a specific antibody. These studies also do not speak to other immune system-related actions that might underlie the benefits (or adverse effects) of active immunization. Nonetheless, given the preliminary data that anti-A $\beta$  immunotherapy may stabilize cognitive function in Alzheimer's patients (Hock et al., 2003) and the consistent reversal of the phenotype found in APP transgenic mice by such approaches, these results support further development of optimal strategies for using anti-A $\beta$  immunotherapy as a treatment for Alzheimer's dementia.

## References

- Banks WA, Terrill BJ, Farr SA, Robinson SM, Nonaka N, Morley JE (2002) Passage of amyloid  $\beta$  protein antibody across the blood-brain barrier in a mouse model of Alzheimer's disease. *Peptides* 25:2223–2226.
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang L, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberberg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, et al. (2000) Peripherally administered antibodies against amyloid  $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nat Med* 6:916–919.
- Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, Guido T, Hoenow K, Hu K, Johnson-Wood K, Khan K, Kholodenko D, Lee C, Lee M, Motter R, Nguyen M, Reed A, Shen D, Tang T, Vasquez N, et al. (2003) Epitope and isotype specificities of antibodies to beta-amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci USA* 100:2023–2028.
- Das P, Howard V, Loochbrook N, Dickson D, Murphy MP, Golde TE (2003) Amyloid  $\beta$  immunization effectively reduces amyloid deposition in TgCRGAM- $\beta$ —knock-out mice. *J Neurosci* 23:8532–8538.
- DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (2001) Peripheral anti-A $\beta$  antibody alters CNS and plasma A $\beta$  clearance and decreases brain A $\beta$  burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:8850–8855.
- Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu X, Wu X, Holtzman DM, Paul SM (2002) Immunization reverses memory deficits without reducing brain A $\beta$  burden in Alzheimer's disease model. *Nat Neurosci* 5:452–457.
- Ferrer J, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa I (2004) Neuroinflammation and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 14:11–20.
- Giesner JE, Heiken H, Tamini A, Schmidt RE (1998) The IgG Fc receptor family. *Ann Hematol* 76:231–248.
- Gordon MN, Holcomb LA, Jantzen PT, DiCarlo G, Wilcock D, Boyett KW, Connor K, Melachroinou I, O'Callaghan JP, Morgan D (2002) Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1 + APP mouse. *Exp Neurol* 173:183–195.
- Hock C, Konietzko U, Papassotiropoulos A, Wollmer A, Streffer J, Von Rutz RC, Davey G, Moritz E, Nitsch RM (2002) Generation of antibodies specific for beta-amyloid by vaccination of patients with Alzheimer's disease. *Nat Med* 8:1270–1275.
- Hock C, Konietzko U, Streffer JR, Tracy I, Signorelli A, Muller-Tillmann B, Lemke U, Henke K, Moritz E, Garcia E, Wollmer MA, Umbriech D, de Quervain DJ, Hofmann M, Maddelein A, Papassotiropoulos A, Nitsch RM (2003) Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 38:547–554.
- Holcomb LA, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 4:97–100.
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang L, Cole G (1996) Correlative memory deficits, A $\beta$  elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102.
- Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, George-Hyslop P, Westaway D (2000) A beta peptide immunization reduces behavioural impairment and plaques in a mouse model of Alzheimer's disease. *Nature* 408:979–982.
- Kotilinek LA, Bales KR, Westerman M, Kawarabayashi T, Younkin L, Hyman BT, Younkin S, Ashe KH (2002) Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* 22:6331–6335.
- Lemere CA, Spooner FT, Lafrancois J, Molest B, Mori C, Ieverone JF, Matsuo Y, Taylor JW, DeMattos RB, Holtzman DM, Clements JD, Selkoe DJ, Duff KE (2003) Evidence for peripheral clearance of cerebral A $\beta$  protein following chronic, active A $\beta$  immunization in PSAPP mice. *Neurobiol Dis* 10–18.
- Li Q, Cao C, Chackarian B, Schiller J, Gordon M, Morgan D (2004) Overcoming antigen masking of anti-A $\beta$  antibodies reveals breaking of B cell tolerance by virus-like particles in A $\beta$  immunized amyloid precursor protein transgenic mice. *BMC Neurosci* 5:21.
- Liu B, Wang K, Cao JM, Mandavilli B, Wang JY, Hong JS (2001) Molecular consequences of activated microglia in the brain: overactivation induces apoptosis. *J Neurochem* 77:182–189.
- Manuelidis L (1998) Vaccination with an attenuated Creutzfeldt-Jakob disease strain prevents expression of a virulent agent. *Proc Natl Acad Sci USA* 95:2520–2525.

- McMichael AJ, Hanke T (2003) HIV vaccines 1983–2003. *Nat Med* 9:874–880.
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982–985.
- Scheek D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson Wood K, Khan K, Kholodenko D, Lee M, Lee Z, Lieberburg I, Motter R, Mutter L, Soriano I, Shopp G, Vasquez N, Vandewert C, et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–177.
- Seubrock TI, Bloom JK, Iglesias M, Spooner ET, Walsh DM, Lemere CA (2004) Species-specific immune response to immunization with human vs. rodent A $\beta$  peptide. *Neurobiol Aging*, in press.
- Sigurdsson FM, Sr MS, Li R, Scholtzova H, Kascak RJ, Kascak R, Carp R, Meeker HC, Frangione B, Wisniewski T (2003) Anti-prion antibodies for prophylaxis following prion exposure in mice. *Neurosci Lett* 336:185–187.
- Solomon B, Koppel R, Frenkel D, Hanan-Aharon E (1997) Disaggregation of Alzheimer beta-amyloid by site-directed monoclonal antibodies. *Proc Natl Acad Sci USA* 94:4109–4112.
- Wilcock DM, Gordon MN, Ugen KE, Gottschall PE, DiCarlo G, Dickey C, Boyett KW, Jantzen PJ, Connor KE, Melachroinou J, Hardy J, Morgan D (2001) Number of A $\beta$  inoculations in APP+PS1 transgenic mice influences antibody titers, microglial activation, and congophilic plaque levels. *DNA Cell Biol* 20:731–736.
- Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, Gordon MN, Morgan D (2003) Intracranially administered anti-A $\beta$  antibodies reduce  $\beta$ -amyloid deposition by mechanisms independent of and associated with microglial activation. *J Neurosci* 23:3745–3751.
- Wilcock DM, Munireddy SK, Rosenthal A, Ugen KE, Gordon MN, Morgan D (2004) Microglial activation facilitates A $\beta$  plaque removal following intracranial anti-A $\beta$  antibody administration. *Neurobiol Dis* 15:11–20.

# Intracranially Administered Anti- $A\beta$ Antibodies Reduce $\beta$ -Amyloid Deposition by Mechanisms Both Independent of and Associated with Microglial Activation

Donna M. Wilcock,<sup>1</sup> Giovanni DiCarlo,<sup>1</sup> Debbi Henderson,<sup>1</sup> Jennifer Jackson,<sup>1</sup> Keisha Clarke,<sup>1</sup> Kenneth E. Ugen,<sup>2</sup> Marcia N. Gordon,<sup>1</sup> and Dave Morgan<sup>1</sup>

Departments of <sup>1</sup>Pharmacology and <sup>2</sup>Medical Microbiology and Immunology, Alzheimer's Research Laboratory, University of South Florida, Tampa, Florida 33612

Active immunization against the  $\beta$ -amyloid peptide ( $A\beta$ ) with vaccines or passive immunization with systemic monoclonal anti- $A\beta$  antibodies reduces amyloid deposition and improves cognition in APP transgenic mice. In this report, intracranial administration of anti- $A\beta$  antibodies into frontal cortex and hippocampus of Tg2576 transgenic APP mice is described. The antibody injection resulted initially in a broad distribution of staining for the antibody, which diminished over 7 d. Although no loss of immunostaining for deposited  $A\beta$  was apparent at 4 hr, a dramatic reduction in the  $A\beta$  load was discernible at 24 hr and was maintained at 3 and 7 d. A reduction in the thioflavine-S-positive compact plaque load was delayed until 3 d, at which time microglial activation also became apparent. At 1 week after the injection, microglial activation returned to control levels, whereas  $A\beta$  and thioflavine-S staining remained reduced. The results from this study suggest a two-phase mechanism of anti- $A\beta$  antibody action. The first phase occurs between 4 and 24 hr, clears primarily diffuse  $A\beta$  deposits, and is not associated with observable microglial activation. The second phase occurs between 1 and 3 d, is responsible for clearance of compact amyloid deposits, and is associated with microglial activation. The results are discussed in the context of other studies identifying coincident microglial activation and amyloid removal in APP transgenic animals.

**Key words:** Alzheimer's disease;  $A\beta$ ; antibody; immunization; intracranial; phagocytosis

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive deficits. There are several pathological characteristics to the disease process, including congophilic amyloid plaques that contain the  $\beta$ -amyloid peptide ( $A\beta$ ) and intracellular inclusions of neurofibrillary tangles that consist of hyperphosphorylated tau protein. Another characteristic of AD is the initiation and proliferation of a brain-specific inflammatory response that consists of activated microglia and astrocytes. Amyloid deposition is thought to be the key step in the pathogenesis of AD (Selkoe, 1991; Hardy and Selkoe, 2002); this is why development of potential therapies focuses on clearance of amyloid.

Vaccination with  $A\beta_{1-42}$  was first described by Schenk et al. (1999). Their report demonstrated that active immunization with  $A\beta_{1-42}$  in the PDAPP transgenic mouse reduced levels of  $A\beta$  deposits dramatically. This immunization protected APP+PS1 transgenic mice (Morgan et al., 2000) and TgCRND8 transgenic mice (Janus et al., 2000) from memory deficits. More recent studies showed that treatment with a passive immunization regimen that consisted of anti- $A\beta$  antibodies resulted in a dramatic reduction in  $A\beta$  (Bard et al., 2000; DeMattos et al., 2001) and reversal

of memory deficits (Dodart et al., 2002; Kotilinek et al., 2002) in the PDAPP mouse.

In this experiment, we show that intracranially administered anti- $A\beta$  antibodies have both an early microglia-independent and a later, possibly microglia-dependent mechanism of action.  $A\beta$  levels were dramatically reduced 24 hr after administration in the absence of microglial activation. However, 72 hr after antibody administration, thioflavine-S-positive compact plaques were reduced concomitant with a striking activation of microglia.

## Materials and Methods

Transgenic Tg2576 APP mice (Hsiao et al., 1996) were obtained after breeding of Tg2576 APP mice with line 5.1 PS1 mice (Duff et al., 1996), which yielded four different genotypes: nontransgenic, transgenic APP, transgenic PS1, and doubly transgenic APP+PS1 mice. Animals were provided food and water *ad libitum* and were kept on a 12 hr light/dark cycle; they were housed in groups if possible until before the surgery, when they were all singly housed until they were killed. We used two cohorts of mice in this study, the first cohort of 19-month-old APP mice ( $n = 16$ ) and the second cohort of 16-month-old APP mice ( $n = 22$ ).

Mice from the first cohort all received anti- $A\beta$  antibodies (Bioscience, Camarillo, CA; mouse anti- $A\beta$  IgG<sub>1</sub>, recognizing amino acids 1–16). Mice from the second group were assigned to groups that received anti- $A\beta$  antibodies, control antibody (anti-HIV, ID6; K. Ugen, Department of Medical Microbiology and Immunology, University of South Florida, Tampa, FL) ( $n = 5$ ), or vehicle (0.02% thimerosal in PBS; Sigma-Aldrich, St. Louis, MO) ( $n = 5$ ). All mice were injected in both the frontal cortex and hippocampus of the right hemisphere, whereas the left hemisphere remained untreated as an internal control. Those mice that received anti- $A\beta$  antibodies were assigned survival times of 4 ( $n = 5$ ), 24 ( $n = 7$ ), 72 ( $n = 8$ ), or 168 ( $n = 6$ ) hr. Mice receiving either control

Received Sept. 17, 2002; revised Jan. 21, 2003; accepted Jan. 29, 2003.

This work was supported by National Institutes of Aging, National Institutes of Health Grants AG15490 (M.N.G.), AG14478 (D.M.), and AG20227 (K.E.U.). D.M. is a Bergamini Scholar in Alzheimer's Disease Research.

Correspondence should be addressed to Dave Morgan, 12901 Bruce B. Downs Boulevard, MDC 9, Tampa, FL 33612. E-mail: dmorgan@usf.edu.

Copyright © 2003 Society for Neuroscience 0270-6474/03/233745-07\$15.00/0

antibody or vehicle were examined after a 72 hr survival time. A third group of untreated 17-month-old APP mice ( $n = 5$ ) were killed without having been injected or manipulated to assess differences between the right and left sides of the brain.

On the day of surgery, the mice were weighed, anesthetized with isoflurane, and placed in a stereotaxic apparatus (51603 dual manipulator laboratory standard; Stoelting, Wood Dale, IL). A midsagittal incision was made to expose the cranium, and two burr holes were drilled with a dental drill over the right frontal cortex and hippocampus to the following coordinates: cortex, anteroposterior,  $-1.5$  mm; lateral,  $-2.0$  mm; hippocampus, anteroposterior,  $-2.7$  mm; lateral,  $-2.5$  mm, all taken from bregma. A 26 gauge needle attached to a 10  $\mu$ l syringe (Hamilton, Reno, NV) was lowered 3 mm ventral to bregma, and a 2  $\mu$ l injection was made over a 2 min period. The incision was cleaned with saline and closed with surgical staples.

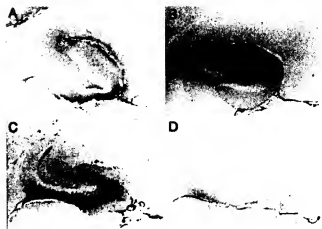
On the day they were killed, the mice were overdosed with pentobarbital 100 mg/kg (Nembutal sodium solution; Abbott Laboratories, North Chicago, IL) and perfused intracardially with 25 ml of 0.9% sodium chloride and 50 ml of freshly prepared 4% paraformaldehyde, pH 7.4. The brains were collected and postfixed for 24 hr in 4% paraformaldehyde. The brains were then incubated for 24 hr in 10, 20, and 30% sucrose sequentially to cryoprotect them. Horizontal sections of 25  $\mu$ m thickness were then collected with a sliding microtome and stored at 4°C in Dulbecco's PBS buffer with sodium azide to prevent microbial growth. Six to eight sections  $\sim 100$   $\mu$ m apart were selected that spanned the injection site and were stained by free-floating immunohistochemistry methods for total A $\beta$  (rabbit antiserum primarily reacting with the N terminus of the A $\beta$  peptide; 1:10,000), CD45 (Serotec, Raleigh, NC; 1:3000), and major histocompatibility complex class II (MHC-II; BD Pharmingen, Palo Alto, CA; 1:3000) as described previously (Gordon et al., 2002). For immunostaining, some sections were omitted from the primary antibody to assess nonspecific immunohistochemical reactions. Immunohistochemical methods were used to stain for the injected antibody with anti-mouse IgG conjugated to horseradish peroxidase (Sigma-Aldrich; 1:1000). Adjacent sections were mounted on slides and stained with 4% thioflavine-S (Sigma-Aldrich) for 10 min. Selected sections stained for CD45 were counterstained for Congo red (Sigma-Aldrich) to detect amyloid deposits on these sections.

The immunohistochemical reaction product on all stained sections was measured with a viduometric V150 image analysis system (Oncor, San Diego, CA) in the injected area of cortex and hippocampus and corresponding regions on the contralateral side of the brain. Data are presented as the average ratio of injected side to noninjected side for A $\beta$ , thioflavine-S, and CD45, whereas data for MHC-II are expressed as area occupied by positive stain, because many values on the contralateral side were close to zero.

To assess possible treatment-related differences, the measurement for either cortex or hippocampus of each subject were analyzed by ANOVA with StatView software version 5.0.1 (SAS Institute Inc., Raleigh, NC) followed by Fisher's least significant difference means comparisons.

## Results

Immunohistochemistry against mouse IgG was performed to trace the diffusion of anti-A $\beta$  antibodies after injection into the hilus of the dentate gyrus. The injected anti-A $\beta$  antibody showed diffuse distribution throughout the entire hippocampus at 4 hr, with a focal concentration in the outer molecular areas of the dentate and Ammons' horn near the hippocampal fissure (Fig. 1A). By 24 hr, the diffuse pattern remained broad, but the focal concentration began shifting toward the granule cell layers of the dentate gyrus (Fig. 1B). At 72 hr, staining for the injected antibody was lighter and became concentrated at the granule cell layer of the dentate gyrus (Fig. 1C). Interestingly, by the 1 week time point, the injected antibody staining had cleared for the most part, with some residual staining in the outer molecular layer of the ventral (ventricular) blade of the dentate gyrus and



**Figure 1.** Time course of injected anti-A $\beta$  antibody distribution in the hippocampus from 4 hr to 7 d. Immunohistochemical staining is shown for the injected antibody in the hippocampus at 4 (A), 24 (B), 72 (C), and 168 (D) hr. Orientation and locations of hippocampal subregions are as in Figure 2D. Magnification, 40 $\times$ . Scale bar, 120  $\mu$ m. A high-resolution color version of this micrograph can be obtained by e-mail from D. Morgan (dmorgan@phsc.usf.edu).

the glial limitans. A similar time course of staining was seen in the frontal cortex (data not shown).

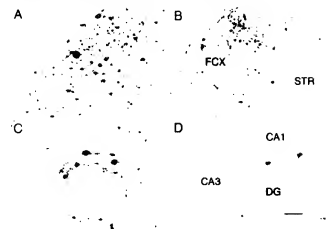
A $\beta$  immunohistochemistry in APP transgenic mice resembled that reported previously by others and ourselves (Hsiao et al., 1996; Gordon et al., 2002). In both cortex (Fig. 2A) and hippocampus (Fig. 2C), there were a few intensely stained deposits and a number of smaller, less intensely stained deposits. In previous work, we found the intensely stained A $\beta$  deposits were usually also stained with thioflavine-S or Congo red (Holcomb et al., 1998; Gordon et al., 2001), which indicates they were analogous to compact deposits containing fibrillar amyloid, whereas the less intense deposits were analogous to diffuse, nonfibrillar deposits observed commonly in AD tissue. Whereas the deposits were distributed fairly uniformly within the cortex, in the hippocampus they were concentrated in the outer molecular layers of the dentate gyrus and Ammons' horn (Fig. 2C). The subiculum also appeared more rich in deposits than other areas.

The injection of anti-A $\beta$  antibody into brain did not result in a rapid loss of signal in postmortem immunohistochemical reactions, because we did not observe a change in A $\beta$  staining 4 hr after injection in either cortex (Fig. 3A) or hippocampus (Fig. 3B). However, A $\beta$  staining was reduced at the injection sites in frontal cortex and hippocampus 24 hr after administration of anti-A $\beta$  antibody (Fig. 2B,D, respectively) and remained reduced to roughly the same extent through the 1 week time point (Fig. 3). The reduction in the frontal cortex was 60% compared with both the 4 hr time points and the two control groups of vehicle and anti-HIV antibody (Fig. 3A;  $p < 0.001$ ). The reduction in the hippocampus was 50% compared with the 4 hr time points and the control groups (Fig. 3B;  $p < 0.005$ ).

An interesting phenomenon was that the ratio of A $\beta$  staining on the right to left sides in untreated mice was  $\geq 1$ , which indicates more A $\beta$  deposition on the right side than the left (Fig. 3). It appears that this pattern of A $\beta$  deposition is a consistent property of the APP mice. The A $\beta$  distribution seen in the mice administered control injections at 3 d and anti-A $\beta$  antibody at the 4 hr time point is the typical distribution found in APP transgenic mice of this age.

As expected, considerably fewer deposits were stained with thioflavine-S than by A $\beta$  immunohistochemistry. Nonetheless, the regional distribution of these deposits approximately paral-





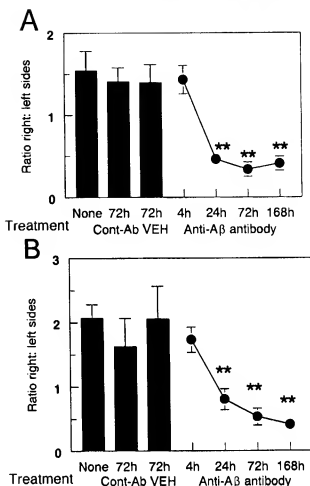
**Figure 2.** Reduction in A $\beta$  immunohistochemistry 1 d after anti-A $\beta$  antibody injections. Immunohistochemical staining is shown for A $\beta$  in the frontal cortex (A, B) and hippocampus (C, D). A and C are from an animal injected with control antibody, whereas animals for which stains are shown in B and D received the anti-A $\beta$  antibody. Magnification, 40 $\times$ . Scale bar, 120  $\mu$ m. B, FCX, frontal cortex; STR, striatum; D, CA1, cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus. A high-resolution color version of this micrograph can be obtained by e-mail from D. Morgan (dmorgan@hsx.usf.edu).

leled that of A $\beta$ -positive deposits in the cortex and hippocampus (Fig. 4A,C). In contrast to the A $\beta$ , thioflavine-S-positive staining at the injection site was not reduced until 72 hr after administration of anti-A $\beta$  antibody (Fig. 4B,D) and remained reduced at the 1 week time point (Fig. 5). The reduction in frontal cortex was 80% compared with the 4 and 24 hr time points and the control groups (Fig. 5A;  $p < 0.001$ ). The reduction in hippocampus was 60% compared with both the 4 and 24 hr time points and the control groups (Fig. 5B;  $p < 0.005$ ).

In untreated mice, activated microglia stained with CD45 or MHC-II antibodies were found only in the immediate periphery of compacted plaques. In the injected control groups, some microglial activation was detected at the 72 hr survival time by CD45 antibodies, and this was restricted primarily to the injection site (Fig. 6A,C, arrows; quantified in Fig. 7). Very little staining for CD45 was detected on the uninjected side of the brain, which led to inflated right-to-left ratios with relatively small increases in staining. MHC-II had a lower overall level of expression than that of CD45 and was mostly unaffected in mice administered control injections (Fig. 8A,C; quantified in Fig. 9).

In contrast, 72 hr after the injection of anti-A $\beta$  antibodies, the microglial activation detected with CD45 antibodies was more widespread, detected not only at the injection site but also away from the injection site in the frontal cortex (Fig. 6B) and throughout the dentate gyrus, with a concentration within the granule cell layer at the 72 hr time point (Fig. 6D). MHC-II staining revealed a similar pattern, although not as extensive as that found with CD45 staining (Fig. 8B,C).

Quantification of these results indicated that the injection of anti-A $\beta$  antibodies increased expression of the microglial marker CD45 significantly only at the 72 hr time point compared with all other time points and control groups in both cortex (Fig. 7A;  $p < 0.005$ ) and hippocampus (Fig. 7B;  $p < 0.005$ ). Also, the injection of anti-A $\beta$  antibodies increased the expression of the microglial marker MHC-II at the 72 hr time point compared with all other time points and control groups in both cortex (Fig. 9A;  $p < 0.01$ ) and hippocampus (Fig. 9B;  $p < 0.005$ ). The expression of CD45 and MHC-II in the frontal cortex at the anti-A $\beta$  injection site increased more than eightfold over that of all other time points,



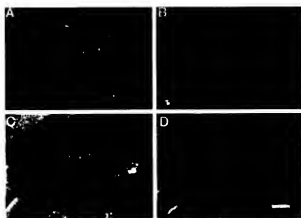
**Figure 3.** Quantification of reduced A $\beta$  load after anti-A $\beta$  antibody injections. Data are expressed as the ratio of A $\beta$  staining in the injected hemisphere to the control hemisphere. The three bars on the left indicate the A $\beta$  load in the untreated group (None) and the vehicle (VEH) and anti-HIV antibody (Cont-Ab) groups at 72 hr. The line shows the ratio of A $\beta$  immunohistochemical staining at 4, 24, 72, and 168 hr survival times. Reduced A $\beta$  load was observed in the frontal cortex (A) and hippocampus (B) at 24, 72, and 168 hr compared with 4 hr and both control groups (\* $p < 0.005$ ).

including 1 week, and both of the control groups. The expression of CD45 in the hippocampus at the anti-A $\beta$  injection site increased more than twofold, whereas the increase in expression of MHC-II was more than eightfold. As in our previous work, there was considerable variability among samples with both microglial markers; however, all anti-A $\beta$ -injected animals had values that were greater than the means for the control groups.

There were few remaining amyloid deposits near the injection sites in the anti-A $\beta$  antibody-injected mice at 72 hr (Figs. 4, 5). These residual deposits were relatively faint when stained with Congo red and were contacted by rounded, CD45-positive microglial cells (Fig. 10B). In contrast, the more abundant amyloid deposits on the contralateral side and in the control animals were contacted by microglia with long processes that were stained for CD45, whereas the cell body stained only faintly for this marker of microglial activation (Fig. 10A).

## Discussion

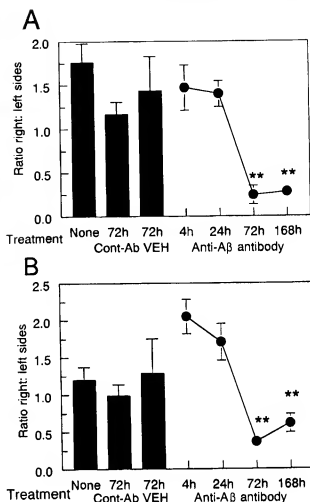
Intracranial anti-A $\beta$  antibody injections reduced A $\beta$  load substantially in the vicinity of the injection in both anterior cortex and hippocampus over a 7 d time frame. By 4 hr after the injection,



**Figure 4.** Reduction in thioflavine-S staining 3 d after anti-A $\beta$  antibody injections. Thioflavine-S staining is shown in frontal cortex (A, B) and hippocampus (C, D). Mice in A and C received control antibody, whereas those in B and D received anti-A $\beta$  antibody. Magnification, 40 $\times$ . Scale bar, 120  $\mu$ m. Orientation and locations of major subregions are as in Figure 2, B and D. A high-resolution color version of this micrograph can be obtained by e-mail from D. Morgan (dmorgan@hsc.usf.edu).

tion, there was a broad distribution of injected antibody that filled a volume of  $\sim 0.5$  mm<sup>3</sup>, as estimated from anti-IgG immunohistochemistry. In addition to the broad pattern of diffusion, the antibody was concentrated in the outer molecular layers of Ammon's horn and the dentate gyrus, a zone that mostly overlaps with the distribution of A $\beta$  staining in transgenic mice of this age (Fig. 2C). Thus, it appears the injected antibody was binding to *in situ* A $\beta$  at this early time point but was also spread throughout the hippocampus. By 24 hr, there was a reduction in the A $\beta$  immunostaining in the vicinity of the antibody injection in both cortex and hippocampus. This reduction in A $\beta$  load is unlikely to be an artifact caused by the injected antibody masking the epitope of the primary antibody used for immunohistochemistry, because the reduced load was not detected 4 hr after administration, and by 24 hr, the injected IgG appeared to be concentrated closer to the granule cell region than the outer molecular layer in the hippocampus. Furthermore, the stoichiometry of injected antibody (13 pmol) to A $\beta$  in deposits (estimated at 250 pmol in 0.5 mg; Chapman et al., 1999) is likely too low to interfere substantially with the histochemical reaction. This early reduction in A $\beta$  load occurred in the absence of the expression of microglial activation markers CD45 and MHC-II. Although this does not preclude some rapid response of the microglia, it does suggest that the role of microglia is qualitatively different at this early postsurvival time from when markers of activation are being expressed.

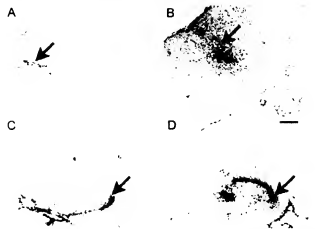
Between 24 and 72 hr after injection of anti-A $\beta$  antibodies, there were parallel reductions in fibrillar amyloid deposits detected by thioflavine-S and increases in microglial activation evaluated by CD45 and MHC-II staining. Although control injections of anti-HIV antibody and vehicle caused some elevation of the CD45 marker, activation was restricted to the immediate vicinity of the injection site and was likely caused by mechanical injury associated with needle insertion and fluid compression of the tissue. Occasionally, in the anti-A $\beta$  antibody-injected mice, some remaining wisps of amyloid could be found in the vicinity of the antibody injection at 72 hr, and these were in contact with rounded CD45-immunopositive cells suggestive of phagocytic microglia or macrophages. Also at the 72 hr time point, there was a concentration of staining for both the injected antibody and the microglia near the granule cell layer of the dentate gyrus. The temporal association of fibrillar amyloid loss with microglial ac-



**Figure 5.** Anti-A $\beta$  antibody injections result in a reduction of thioflavine-S-positive plaques. Data are expressed as the ratio of thioflavine-S staining in the injected hemisphere to the control hemisphere. The three bars show thioflavine-S-positive staining in the untreated group (None) and the vehicle (VEH) and anti-HIV antibody (Cont-A $\beta$ ) groups at 72 hr. The line shows the ratio of thioflavine-S staining at 4, 24, 72, and 168 hr survival times. Reduced thioflavine-S staining was observed in the frontal cortex (A) and hippocampus (B) at 72 and 168 hr compared with 4 and 24 hr and both control groups (\*\* $p < 0.005$ ).

tivation suggests some causal role for microglial activation in this process. One possibility is that between 1 and 3 d, activated microglia near the deposits in the outer molecular layer phagocytosed opsonized amyloid via Fc receptor- or complement-mediated mechanisms and migrated toward the granule cell layer. CD45-positive microglia could be detected readily in the outer molecular layer near the fissure at 3 d, although they were concentrated most heavily near the granule cell layer at that time point (Fig. 6D). A second option is that after dissolution of the A $\beta$  deposits, the antibodies diffused to the granule cell region independently of the microglia. Possibly, the fibrillar deposits simply required more time to dissolve than the more diffuse material. More detailed time course studies of the period between 1 and 3 d coupled with immunoelectron microscopy will likely be required to decide between these options. Remarkably, the microglial activation was terminated rapidly and returned to normal levels by the 1 week time point in conjunction with a significant reduction in staining for the injected IgG and A $\beta$ .

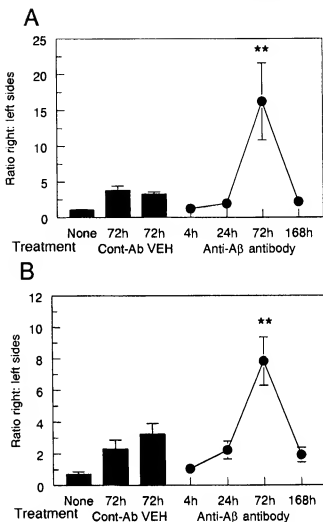
An accumulating body of evidence finds an association between microglial activation and amyloid reductions in transgenic



**Figure 6.** CD45 immunohistochemistry is increased 3 d after anti-A $\beta$  antibody injections. CD45 immunohistochemistry is shown in frontal cortex (A, B) and hippocampus (C, D). Mice in A and C received control antibody, whereas those in B and D received anti-A $\beta$  antibody. Magnification, 40 $\times$ . Scale bar, 120  $\mu$ m. Arrows indicate the site of injection identified from the needle tract. A high-resolution color version of this micrograph can be obtained by e-mail from D. Morgan (dmorgan@hsc.usf.edu).

mouse models of amyloid deposition. Schenk et al. (1999) noted in the first study evaluating A $\beta$  vaccines that the clearance of amyloid was associated with enhanced microglial activity around the remaining deposits. Wilcock et al. (2001) mostly confirmed this observation in a different transgenic model. Nakagawa et al. (2000) unexpectedly found that fluid percussion injury activates microglia and results in reduced amyloid deposition as mice grow older. Lim et al. (2001) noted that transgenic mice treated with curcumin had a reduced amyloid load but an increase in the activation state of microglia surrounding plaques. Similarly, Jantzen et al. (2002) found a reduced amyloid load in transgenic mice treated with a nitric oxide-releasing nonsteroidal anti-inflammatory drug, NCX-2216, which was also associated with increased microglial activation. Wyss-Coray et al. (2001) found that the crossing of APP transgenic mice with mice that overexpressed TGF- $\beta$  led to increased microglial activation and reduced amyloid loads. Conversely, Wyss-Coray et al. (2002) found that blocking of complement activation with soluble complement receptor-related protein Y overexpression diminished the microglial reaction in APP transgenic mice and led to elevated amyloid loads. DiCarlo et al. (2001) attempted to activate microglia directly by injecting lipopolysaccharide and found that this was associated with clearance of A $\beta$  in the vicinity of the injection. However, Qiao et al. (2001) injected lipopolysaccharide chronically into young transgenic mice before normal amyloid deposition and found that it could stimulate A $\beta$  deposition. It is also the case that careful serial section electron microscopy failed to detect internalized amyloid in microglia associated with amyloid deposits in untreated APP23 transgenic mice (Stalder et al., 2001), although mice treated to provoke microglial activation have yet to be examined. Nonetheless, there is a growing body of literature that associates the activation of microglia with a reduction in A $\beta$  deposition in the transgenic mouse models.

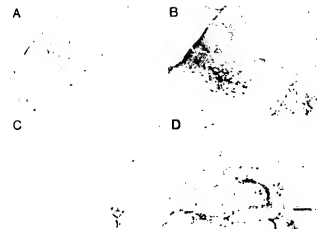
A number of studies have demonstrated that cultured microglial cells are capable of internalizing A $\beta$ 1–42 aggregates (Paresce et al., 1996; Webster et al., 2001). A $\beta$  can also be cleared from unfixed brain sections by anti-A $\beta$  antibodies in a microglia-dependent manner (Bard et al., 2000). Direct imaging of amyloid deposits *in vivo* by multiphoton microscopy has shown clearance of plaque after application of an anti-A $\beta$  antibody in association



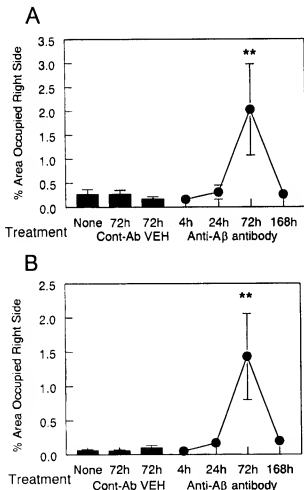
**Figure 7.** Anti-A $\beta$  antibody injections result in increased CD45 immunohistochemistry 3 d after injection. Data are expressed as the ratio of CD45 staining in the injected hemisphere to the control hemisphere. The three bars indicate CD45 expression in the untreated group (None) and the vehicle (VEH) and anti-HIV antibody (Cont-A $\beta$ ) groups at 72 hr. The line shows the ratio of CD45 staining at 4, 24, 72, and 168 hr survival times. Increased CD45 staining was observed in the frontal cortex (A) and hippocampus (B) at 72 hr compared with 4, 24, and 168 hr and both control groups (\* $p$  < 0.005).

with an upregulation of activated microglia (Bacskaï et al., 2001). Suggested alternative mechanisms to microglial phagocytosis include a physical interaction between antibody and A $\beta$  that results in disaggregation of deposits, which was demonstrated *in vitro* with monoclonal anti-A $\beta$  antibodies (Solomon, 2001). Consistent with this idea, Bacskaï et al. (2002) demonstrated recently that F(ab')<sub>2</sub> fragments prepared from an anti-A $\beta$  antibody reduced amyloid deposits as effectively as the intact antibody when applied topically to the cortex of transgenic mice through a craniotomy. Although there was no measurement of microglial activation in this study, it is plausible that this occurred in the absence of microglial involvement. This antibody-mediated dissolution hypothesis is consistent with the early phase of A $\beta$  reduction described here and may still be found to be responsible for the second phase of fibrillar deposit reduction.

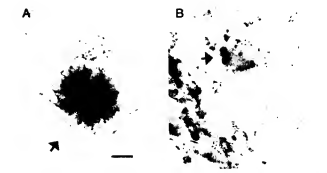
A major unresolved question is how this antibody-mediated clearance of A $\beta$  might apply to the human condition. Increasingly, AD has been argued to involve inflammation as a component of its pathogenesis (McGeer and McGeer, 2001). The early



**Figure 8.** MHC-II immunohistochemistry is increased 3 d after anti-A $\beta$  antibody injections. MHC-II immunohistochemistry is shown in frontal cortex (A, B) and hippocampus (C, D). Mice in A and C received control antibody, whereas those in B and D received anti-A $\beta$  antibody. Magnification,  $40\times$ . Scale bar, 120  $\mu$ m. A high-resolution color version of this micrograph can be obtained by e-mail from D. Morgan (dmorgan@hsc.usf.edu).



**Figure 9.** Anti-A $\beta$  antibody injections result in an increase in MHC-II immunohistochemistry 3 d after injection. Data are expressed as percentage area occupied by MHC-II-positive staining in the injected hemisphere. The three bars indicate MHC-II expression in the untreated (None) group and the vehicle (VEH) and anti-HIV antibody (Cont-Ab) groups at 72 hr. The line shows the amount of MHC-II staining at 4, 24, 72, and 168 hr survival times. Increased MHC-II staining was observed in the frontal cortex (A) and hippocampus (B) at 72 hr compared with 4, 24, and 168 hr and both control groups (\*\* $p < 0.01$ ).



**Figure 10.** Anti-A $\beta$  antibody injections result in rounded microglia in association with remaining congophilic amyloid deposits 3 d after injection. CD45 immunostaining counterstained with Congo red is shown in the hippocampus at the 72 hr time point. A, Typical intensely stained congophilic deposit surrounded by CD45 immunostained microglial processes, with faintly stained somata (arrow). B, Faintly stained congophilic deposit in the anti-A $\beta$  antibody-injected hippocampus. Note the two rounded intensely CD45-positive cells in contact with the faintly stained deposit (arrow). Magnification,  $600\times$ . Scale bar, 833  $\mu$ m.

stages of the A $\beta$  vaccine trials resulted in a small fraction of patients developing adverse reactions consistent with inflammation of the CNS, presumably including microglial activation (Hock et al., 2002; Schenk and Yednock, 2002). Although adverse reactions to immunotherapy have been rare in the transgenic models (Pfeifer et al., 2002), it remains the best experimental system in which to understand the different components of the immune reactions to vaccines and to identify those components that cause adverse outcomes. Certainly, identification of immunotherapies that avoid the problem of deleterious CNS inflammation will be necessary if this treatment approach is to find use in the clinical setting. A better understanding of the mechanisms of antibody-mediated clearance of A $\beta$  in the transgenic models of amyloid deposition should benefit this effort.

## References

- Bacskai BJ, Kajdasz ST, Christie RL, Carter C, Games D, Seubert P, Schenk D, Hyman BT (2001) Imaging of amyloid-beta deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. *Nat Med* 7:369–372.
- Bacskai BJ, Kajdasz ST, McLellan ME, Games D, Seubert P, Schenk D, Hyman BT (2002) Non-IC-mediated mechanisms are involved in clearance of amyloid- $\beta$  in vivo by immunotherapy. *J Neurosci* 22:7873–7878.
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberberg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, et al. (2000) Peripherally administered antibodies against amyloid  $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nat Med* 6:916–919.
- Chapman PF, White GL, Jones MW, Cooper Blacketer D, Marshall VL, Irizarry M, Younkin L, Good MA, Bliss TYP, Hyman BT, Younkin SG, Hsiao KK (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci* 2:271–276.
- DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (2001) Peripheral anti-A $\beta$  antibody alters CNS and plasma A $\beta$  clearance and decreases brain A $\beta$  burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:8850–8855.
- DiCarlo G, Wilcock D, Henderson D, Gordon M, Morgan D (2001) Intrahippocampal IPS injections reduce A $\beta$  load in APP+PS1 transgenic mice. *Neurobiol Aging* 22:1007–1012.
- Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu X, Wu X, Holtzman DM, Paul SM (2002) Immunization reverses memory deficits without reducing brain A $\beta$  burden in Alzheimer's disease model. *Nat Neurosci* 5:452–457.
- Duff K, Eckman C, Zehr C, Yu X, Prada CM, Pei Z, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo J, Zink B, Hardy J, Younkin S (1996) Increased amyloid- $\beta$ 42(43) in brains of mice expressing mutant presenilin 1. *Nature* 383:710–713.

- Gordon MN, King DL, Diamond DM, Jantzen PT, Boyett KV, Hope CE, Hatcher JM, DiCarlo G, Gottschall WP, Morgan D, Arendash GW (2001) Correlation between cognitive deficits and A $\beta$  deposits in transgenic APP + PS1 mice. *Neurobiol Aging* 22:377–385.
- Gordon MN, Holcomb LA, Jantzen PT, DiCarlo G, Wilcock D, Boyett KV, Connor K, Melachroinou J, O'Callaghan JP, Morgan D (2002) Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1 + APP mouse. *Exp Neurol* 173:183–195.
- Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297:353–356.
- Hock C, Konczak U, Papassotiropoulos A, Wollmer A, Streffer J, Von Rotz RC, Dayer C, Mottet J, Nitsch RM (2002) Generation of antibodies specific for beta-amyloid by vaccination of patients with Alzheimer disease. *Nat Med* 8:1270–1275.
- Holcomb J, Gordon MN, McGowan F, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zelt C, O'Campo K, Hardy J, Prada CM, Fickman C, Younkin S, Hsiao K, Duff K (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 4:97–100.
- Hsiao K, Chapman P, Nilsen S, Fickman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, A $\beta$  elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102.
- Jantzen PT, Connor KE, DiCarlo G, Wenk GL, Wallace JL, Rojiani AM, Coppola D, Morgan D, Gordon MN (2002) Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroid anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. *J Neurosci* 22:2246–2254.
- Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti BI, Younkin S, Ashe KH (2002) Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* 22:6331–6335.
- Lim GP, Chu T, Yang F, Beech W, Frautschi SA, Cole GM (2001) The curcumin curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci* 21:8370–8377.
- McGeer PL, McGeer EG (2001) Inflammation, autoimmunity and Alzheimer disease. *Neurobiol Aging* 22:799–809.
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982–985.
- Nakagawa Y, Reed L, Nakamura M, McIntosh TK, Smith DH, Saatman KE, Raghupathi R, Clemens J, Saito TC, Lee VM, Trojanowski JQ (2000) Brain trauma in aged transgenic mice induces regression of established A $\beta$  deposits. *Exp Neurol* 163:244–252.
- Paresce DM, Ghosh RN, Maxfield FR (1996) Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. *Neuron* 17:553–565.
- Pfeifer M, Boncristiano S, Bondolfi J, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M (2002) Cerebral hemorrhage after passive anti-A $\beta$  immunotherapy. *Science* 298:1739.
- Qiao X, Cummins DJ, Paul SM (2001) Neuroinflammation-induced acceleration of amyloid deposition in the APPV717F transgenic mouse. *Eur J Neurosci* 14:474–482.
- Schenk DB, Yednock T (2002) The role of microglia in Alzheimer's disease: friend or foe? *Neurobiol Aging* 23:677–679.
- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandeventer C, et al (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–177.
- Selkoe DJ (1991) The molecular pathology of Alzheimer's disease. *Neuron* 6:487–498.
- Solomon B (2001) Immunotherapeutic strategies for prevention and treatment of Alzheimer's disease. *DNA Cell Biol* 20:697–703.
- Stalder M, Deller T, Staufenbiel M, Jucker M (2001) 3D-reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid. *Neurobiol Aging* 22:427–434.
- Wehster SD, Galvan MD, Ferran E, Garzon-Rodriguez W, Gilabre CG, Tenner AJ (2001) Antibody-mediated phagocytosis of the amyloid beta-peptide in microglia is differentially modulated by C1q. *J Immunol* 166:7496–7503.
- Wilcock DM, Gordon MN, Ugen KE, Gottschall PE, DiCarlo G, Dickey C, Boyett KV, Jantzen PT, Connor KE, Melachroinou J, Hardy J, Morgan D (2001) Number of A $\beta$  inoculations in APP + PS1 transgenic mice influences antibody titers, microglial activation, and conophilic plaque levels. *DNA Cell Biol* 20:731–736.
- Wyss-Coray T, Lin C, Yan F, Yu GQ, Rohde M, McConlogue L, Masliah E, Mucke I (2001) TGF-beta1 promotes microglial amyloid-beta clearance and reduces plaque burden in transgenic mice. *Nat Med* 7:612–618.
- Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, Quigg RJ, Masliah E (2002) Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci USA* 99:10837–10842.

of the circadian cycle in mammals. Mutational analyses of other putative clock genes will be essential for unravelling the molecular mechanisms underlying the mammalian circadian clock. These mutants will also provide useful animal models for elucidating the aetiology of and developing treatments for disorders in humans related to the sleep-wake cycle. □

# Methods

**Generation of *mPer2<sup>ΔB1</sup>* mutant mice.** We isolated a genomic clone from a mouse 129SvEvBrd genomic library using a mouse *mPer2* complementary DNA probe. A targeting vector was constructed with PGK-Neo as the positive selection marker and HSVtk as the negative selection marker to delete a 2.1-kilobase (kb) fragment. We used a 6.7-kb *Bgl*II fragment as the 5' homology region and a 4.0-kb *Kpn*I fragment as the 3' homology region. The *HSVtk*, PGK-Neo and vector backbone were from pKO SelectTK, pKO SelectNeo V800 and pKO Scrambler V924 (Lexicon Genetics). Tissue culture, electroporation, mini-Southern blot analysis on embryonic stem cell colonies, generation of chimeric and germline mice and tail DNA genotyping were done as described<sup>12,14</sup>.

**Locomotor activity monitoring and circadian phenotype analysis.** Mice were housed in individual cages equipped with a running wheel in ventilated, light-tight chambers with controlled lighting. Wheel-running activity was monitored by an on-line PC using the Chronobiology Kit (Stanford Software Systems). In the LD cycle, the light was turned on at 7:00 (ZT 0) and off at 19:00 (ZT 12). The switch into constant darkness (DD) was effected by not turning on the light at the usual time. The activity records are double plotted so that each day/cycle's activity is shown both to the right and below that of the previous day/cycle. Activity is plotted in density percentile distribution (Fig. 2) or threshold (Fig. 3) format. For activity counting we used the ACTCNET program of the Chronobiology Kit. To determine the period length, an interval with a 10-day minimum during which the circadian period appeared to be stable on the activity record was analysed with a  $\chi^2$  periodogram<sup>15</sup> using the Stanford Chronobiology Kit. We used Fourier periodogram analysis<sup>15</sup> in the Chronobiology Kit to assess the strength of circadian and/or ultradian rhythmicity.

**In situ hybridization.** Mice were killed by cervical dislocation under ambient light conditions at ZT 6 and ZT 12 and under a 15 W safety red light at ZT 18 and ZT 0/24. Specimen preparation and *in situ* hybridization with *mPer1* and *mPer2* probes were carried out as described<sup>16</sup>. The *mPer2* probe is outside the region deleted in the mutant. The *mPer3* probe was made from an RT-PCR product corresponding to nucleotides 480–824 (AF050182). The *Clock* probe was made from an RT-PCR product corresponding to nucleotides 1352–2080 (AF000998). Tissue was visualized by fluorescence of Hoechst dyed-stained nuclei (blue in Fig. 4).

**Northern blot and RT-PCR analysis.** Tissues were collected and frozen in liquid nitrogen and stored at –80 °C. RNA was prepared with the RNeasy<sup>TM</sup> B RNA isolation kit (TRI-TEST). We performed northern blot analysis on total tissue RNA using denaturing formaldehyde gel. For RT-PCR analysis, first strand cDNA was generated using Moloney reverse transcriptase (BRL-GIBCO) and oligo dT-priming from total liver RNA. An aliquot of the first strand cDNA was then amplified by PCR across the deletion region with the 5' primer CTA CTT GGT CAA GGT GCA AGA G and the 3' primer GGT TTT AAT CTT GCG ACT GG. The RT-PCR products were then sequenced with an internal primer AGG GTA CAC TCG GCG TAT GA.

Received 11 December 1998; accepted 18 May 1999

- Pittendrigh, C. S. Temporal organization: reflections of a Darwinian clock-watcher. *Annu Rev Physiol* **55**, 163–191 (1993).
- Sun, Z. S. et al. *Per1*, a putative mammalian ortholog of the *Drosophila* period gene. *Cell* **90**, 1003–1011 (1997).
- Te, H. et al. Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. *Nature* **380**, 512–516 (1993).
- Allreuther, U., Sun, Z. S., Fache, G. & Lee, C. A differential response of two putative mammalian circadian regulators, *mPer1* and *mPer2*, to light. *eLife* **9**, 1055–1064 (1997).
- Szathmari, P., Zilka, M. J., Weaver, D. R., Kodolowski, J. & B. Reppert, S. M. Two period homologs: circadian expression and protein regulation in the suprachiasmatic nuclei. *Neuron* **19**, 1261–1269 (1997).
- Takumi, T. et al. A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus. *Genes (Dev)* **3**, 167–176 (1998).
- Zilka, M. J., Szechtman, T. P., Weaver, D. R. & B. Reppert, S. M. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron* **20**, 1105–1110 (1998).

- Takumi, T. et al. A light-independent oscillatory gene *mPer1* in mouse SCN and OVLT. *EMBO J* **17**, 4753–4759 (1998).
- Huang, Z. J., Eide, J. & Rosbash, M. PAS is a dimerization domain common to *Drosophila* Period and several mammalian cryptochrome. *Nature* **384**, 259–262 (1991).
- Crosthwaite, S. K., Dunlap, J. C. & Loros, J. J. Neurospore *per-1* and *wee-1* transcription, photoresponses, and the origins of circadian rhythmicity. *Science* **276**, 763–769 (1997).
- King, D. P. et al. Functional cloning of the mouse circadian clock gene. *Cell* **89**, 641–653 (1997).
- Rutula, J. E. et al. CCKLR is a second *Drosophila* PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila* period and timeless. *Cell* **93**, 805–814 (1998).
- Alhade, R., White, N. E., Su, W. V., Hall, J. C. & Rosbash, M. A mutant *Drosophila* homologue of mammalian Clock disrupts circadian rhythms and transcription of period and timeless. *Cell* **75**, 791–804 (1998).
- Ponting, C. P. & Razandi, L. PAS: a multifunctional domain family comes to light. *Curr Biol* **7**, R674–R677 (1997).
- Bracewell, R. *The Hierarchy of Time* (Oxford Univ. Press, New York, 1986).
- Aschoff, J. in *Handbook of Behavioral Neurobiology 4 Biological Rhythms* (ed. Aschoff, J.) 3–10 (Plenum, New York, 1981).
- Bush, N., Impey, S. J. & Kawamura, H. Analysis of sleep-wakefulness rhythms in male rats after suprachiasmatic nucleus lesion and olfactory enucleation. *Brain Res* **122**, 33–47 (1977).
- Vitaterna, M. J. et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* **264**, 719–725 (1994).
- Dowse, H. B. & Rung, J. M. Further evidence that the circadian clock in *Drosophila* is a population of coupled ultradian oscillators. *J Biol Rhythms* **2**, 65–76 (1987).
- Hamblen-Cayle, M. J., Wheeler, D. A., Rutula, J. E., Rosbash, M. & Hall, J. C. Behavior of period-affected rhythm mutants of *Drosophila* in light-dark cycles. *J Insect Behav* **5**, 417–446 (1992).
- Ralph, M. R. & Menaker, M. A mutation of the circadian system in golden hamsters. *Science* **241**, 1223–1227 (1988).
- Hardin, P. E., Hall, J. C. & Rosbash, M. Feedback of the *Drosophila* period gene product on circadian cycling of its messenger RNA levels. *Nature* **343**, 536–540 (1990).
- Dunlap, J. C. Genetics and molecular analysis of circadian rhythms. *Annu Rev Genet* **30**, 579–601 (1996).
- Balsalobre, A., Damiola, E. & Schibler, U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **93**, 929–937 (1998).
- Sangam, A. M. et al. Mammalian circadian autoregulatory loop: A Timeless ortholog and *mPer1* interact and negatively regulate CLOCK/BMAL1-induced transcription. *Mol Cell Biol* **21**, 1101–1113 (1998).
- Bae, K., Lee, C., Schibler, U., Chung, K. Y. & Eide, J. Circadian regulation of the *Drosophila* homologue of the mammalian Clock gene: PER and TIM function as positive regulators. *Mol Cell Biol* **18**, 6142–6151 (1998).
- Martini, M. M., Engelhard, M. J., Su, J. G., Hsu, A. J. & Bradley, A. Alpha-tubulin is a tumour suppressor gene with genomic specificity in mice. *Nature* **360**, 113–119 (1992).
- Ramirez-Solis, R., Davis, A. C. & Bradley, A. Gene targeting in embryonic stem cells. *Methods Enzymol* **225**, 655–678 (1993).
- Schibler, U. G. & Baudry, W. N. The  $\chi^2$  square periodogram: its utility for analysis of circadian rhythms. *J Theor Biol* **72**, 131–160 (1978).
- Allreuther, U., Eichele, G., Helms, J. A. & Lu, H. In *Molecular and Cellular Methods in Developmental Toxicology* (ed. Daston, G. P.) 23–48 (CRC Press, Boca Raton, FL, 1997).

Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

**Acknowledgements.** We thank S. Vambasi, I. Qiu, Y. C. Chell, F. Sheppard and S. Rivera for technical assistance, P. Hastings for comments on the manuscript, J. W. Patk for providing space and facilities for circadian phenotype analysis, and T. Takahashi, Y. Zhan and M. Bauman for helpful discussions. This work was supported by grants from NINDS and NIDA to J. W. Patk, from the Max-Planck Society to E. L., from NIH and the Department of Defense to C. L., and from NIH and the Howard Hughes Medical Institute to A. B. A.B. is an investigator with HHMI.

Correspondence and requests for materials should be addressed to C. L. (e-mail: [ching@bcm.tmc.edu](mailto:ching@bcm.tmc.edu)).

## Immunization with amyloid- $\beta$ attenuates Alzheimer-disease-like pathology in the PDAPP mouse

Daie Schenk, Robin Barbour, Whitney Dun, Grace Gordon, Henry Grajeda, Teresa Guido, Kang Hu, Jiping Huang, Kelly Johnson-Wood, Karen Khan, Dora Kholodenko, Mike Lee, Zhennan Liao, Ivan Lieberburg, Ruth Motter, Linda Mutter, Ferdie Soriano, George Shopp, Nicki Vasquez, Christopher Vandever, Shannan Walker, Mark Wogulis, Ted Yednock, Dora Games & Peter Seubert

Eli Lilly Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, California 94080, USA

Amyloid- $\beta$  peptide (A $\beta$ ) has been a central role in the neuropathology of Alzheimer's disease (AD)<sup>1</sup>. Familial forms of the disease have been linked to mutations in the amyloid precursor protein (APP) and the presenilin genes<sup>2,3</sup>. Disease-linked mutations in these genes result in increased production of the



immune responses against plaque components *per se* are insufficient to prevent or eliminate  $\beta$ -amyloid plaques and neuropathology.

The brains of  $\text{A}\beta_{42}$ -treated mice that contained no amyloid- $\beta$  deposits were also devoid of the dystrophic neurites that characterize the neuritic plaques (Fig. 2; compare c and d). Small numbers of dystrophic neurites were present in the two  $\text{A}\beta_{42}$ -treated mice that had detectable  $\text{A}\beta$  deposits. In contrast, all brains from SAP-injected mice and the two control groups (PBS-injected and untreated mice) had numerous neuritic plaques. Image analyses of the hippocampus demonstrated the virtual elimination of dystrophic neurites in the  $\text{A}\beta_{42}$ -treated mice (median, 0.00%) compared with the PBS recipients (median, 0.28%;  $P = 0.0005$ ).

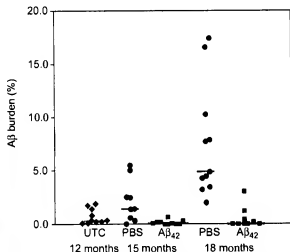
Astrocytosis, another hallmark of plaque-associated pathology in both Alzheimer's disease and PDAPP mice, was dramatically reduced in the brains of all of the  $\text{A}\beta_{42}$ -injected mice (Fig. 2). Brains from mice in all other groups contained numerous clusters of astrocytes that were immunoreactive to glial fibrillary acidic protein (GFAP), a finding typical of  $\text{A}\beta$ -plaque-associated gliosis (Fig. 2c).

Association of amyloid plaques and reactive astrocytes was verified in a subset of GFAP-reacted sections counterstained with thioflavin S. The results of image analyses for the retrosplenial cortex verified that the reduction in astrocytosis was significant, with a median value of 1.55% for mice immunized with  $\text{A}\beta_{42}$  compared with median values of greater than 6% for groups immunized with SAP peptide or PBS, or untreated control mice ( $P = 0.0017$ ).

Sections of the mouse brains were also reacted with a monoclonal antibody specific for MAC-1 (CD11b; Chemicon), a cell-surface marker that is upregulated on activated, plaque-associated microglia. MAC-1 labelling was substantially lower in the brains of mice treated with  $\text{A}\beta_{42}$  compared with the PBS control group, a finding consistent with the lack of a plaque-induced gliosis (not shown).

The almost complete absence of plaques in the brains of  $\text{A}\beta_{42}$ -treated mice indicates that a fundamental mechanism of amyloid plaque formation has been disrupted. Subsequent studies indicate that  $\text{A}\beta$  production itself was unaffected (data not shown).  $\text{A}\beta_{42}$  immunization therefore either prevents deposition and/or enhances the clearance of  $\text{A}\beta$  from the brain. The absence of neuritic and gliotic changes suggests that the  $\text{A}\beta_{42}$ -immunized mice never developed the neurodegenerative lesions that typify the progression of AD-like pathology in this model. The absence of enhanced astrocytosis, in particular, suggests that the processes preventing  $\beta$ -amyloidosis do not in themselves cause appreciable damage to the neuropil.

The above results clearly indicate that  $\text{A}\beta_{42}$  immunization essentially prevents the development of AD-like neuropathology in the PDAPP mouse. It was unclear whether  $\text{A}\beta_{42}$  immunization would improve the pathological outcome if treatment were initiated when a substantial  $\text{A}\beta$  plaque burden already existed. We therefore undertook further experiments in which immunizations with  $\text{A}\beta_{42}$  began at an age when many  $\beta$ -amyloid plaques are already present in the brains of the PDAPP mice. Immunizations were continued during a period when the extent of  $\text{A}\beta$  deposition reaches levels comparable to those of established  $\text{AD}^{11,12}$ . For this study, approximately 11-month-old, heterozygous female PDAPP mice ( $n = 24$ ) were immunized repeatedly with  $\text{A}\beta_{42}$  plus adjuvant, with an injection protocol and schedule equivalent to those used in the young PDAPP mouse study (see Methods). Similar titre responses against  $\text{A}\beta_{42}$  to those seen in younger animals were generated in the older animals. As a negative control, a parallel group of 24 transgenic littermates was immunized with PBS plus adjuvant. One-half of each group was killed at 15 months of age after 4 months' treatment, and the remaining half was killed at 18 months



**Figure 3** Quantitative image analysis of the cortical  $\text{A}\beta$  burden in older PBS- and  $\text{A}\beta_{42}$ -treated mice. Immunization of PDAPP mice was begun at 11 months of age. Amyloid burden was significantly reduced in the  $\text{A}\beta_{42}$  group compared with the PBS controls at both 15 ( $P = 0.003$ ) and 18 ( $P = 0.0002$ ) months of age. The median value of the amyloid burden for each group is shown by the horizontal lines.



**Figure 4** Reduction of cortical  $\text{A}\beta$  deposition in older PDAPP mice immunized with  $\text{A}\beta_{42}$ .  $\text{A}\beta$  deposits in the brains of 12-month-old untreated PDAPP mice and 18-month-old PBS- and  $\text{A}\beta_{42}$ -injected mice with median  $\text{A}\beta$  burdens representative of their respective treatment groups (see Fig. 3). **a**, Distribution of amyloid plaques in the frontal and retrosplenial cortices of a 12-month-old untreated PDAPP mouse; **b**, tyrosinizing the plaque product of both PBS- and  $\text{A}\beta_{42}$ -injected groups at

the start of the study. The plaques are shown at a high magnification in **d**. Compared with the 18-month PBS controls (**b**, **e**),  $\text{A}\beta$  deposits were significantly decreased in the 18-month  $\text{A}\beta_{42}$ -immunized group (**c**, **f**). Most of the cortical  $\text{A}\beta$  in brains of  $\text{A}\beta_{42}$ -injected mice was detected in small extracellular or cell-associated deposits (**f**) compared with the large and numerous extracellular deposits in the PBS group (**e**). Scale bar in **c** corresponds to **a**–**c**. Scale bar in **f** corresponds to **d**, **e**.



of age after 7 months' treatment. Groups of untreated PDAPP mice were also killed at ages 12, 15 and 18 months to serve as age-matched, non-immunized controls. At each time point, brains were examined by image analysis and enzyme-linked immunosorbent assay (ELISA) to determine the magnitude of the amyloid- $\beta$  burden and the extent of neuritic dystrophy, astrogliosis and microgliosis.

Figure 3 shows the results of  $\text{A}\beta_{42}$  treatment on cortical amyloid- $\beta$  burden, determined by quantitative image analysis. The median value of cortical amyloid- $\beta$  burden was 0.28% in untreated, 12-month-old PDAPP mice, a value representative of the plaque load in the experimental mice at the start of the study. At 18 months, the amyloid- $\beta$  burden had increased by more than 17-fold to 4.87% in PBS-treated mice, while  $\text{A}\beta_{42}$ -treated mice had a greatly reduced amyloid burden of only 0.01%. The amyloid- $\beta$  burden was significantly reduced in the  $\text{A}\beta_{42}$  recipients at both 15 months (96% reduction;  $P = 0.003$ ) and 18 months (>99% reduction;  $P = 0.0002$ ). Figure 4 depicts the burden of amyloid- $\beta$  in the 12-month-old PDAPP brain at the start of the experiment (Fig. 4a, d). At 18 months of age, the progression of  $\text{A}\beta$ -plaque pathology was obvious in the PBS group (Fig. 4b, e), but greatly diminished in the  $\text{A}\beta_{42}$ -injected mice (Fig. 4c, f). Compared with the 12-month-old untreated mice, several brains from the  $\text{A}\beta_{42}$  group had fewer diffuse and mature amyloid- $\beta$  deposits at 15 and 18 months, suggesting that the treatment had resulted in the clearance of pre-existing amyloid- $\beta$  deposits (Fig. 4: compare a and d with c and f). Immunohistochemistry with anti-mouse immunoglobulin antibodies showed that the remaining plaques were often decorated

with IgG in  $\text{A}\beta_{42}$ -treated but not in PBS-treated mice at both 15 and 18 months of age (data not shown).

Cortical amyloid- $\beta$  deposition in PDAPP brains begins in the cingulate, frontal and retrosplenial cortices and progresses in a lateral-ventral fashion to sequentially involve the temporal and entorhinal cortices. After 3 months of treatment, amyloid-plaque pathology was diminished in the retrosplenial cortex (Fig. 5a, c) and completely absent in the entorhinal cortex (Fig. 5b, d) of the  $\text{A}\beta_{42}$ -injected mice. The progressive  $\beta$ -amyloidosis that would normally pervade the entorhinal cortex was thus halted by  $\text{A}\beta_{42}$  immunization.

PDAPP mice also invariably develop heavy amyloid- $\beta$  deposition in the outer molecular layer of the hippocampal dentate gyrus<sup>11</sup>. In a number of brains from  $\text{A}\beta_{42}$ -immunized mice, this pattern was considerably altered; the hippocampal deposition no longer contained diffuse amyloid- $\beta$  deposits, and the banded pattern was completely disrupted (Fig. 6). Instead, unusual punctate structures were present that were reactive with anti- $\text{A}\beta$  antibodies, and several appeared to be  $\text{A}\beta$ -containing cells (Fig. 6b). The pattern of apparent cellular labelling produced by the amyloid- $\beta$  antibodies was replicated in adjacent sections by immunolabelling with antibodies directed at major histocompatibility complex (MHC) class II molecules (Fig. 6c). Phenotypically, these cells resembled activated microglia and monocytes and were occasionally found associated with the wall or lumen of blood vessels. They were not immunoreactive with antibodies that recognize T-cell (CD43, CD3e) or B-cell (CD45RA, CD45RB) surface markers (data not shown). No such cells were found in any of the PBS-treated mice.

Amyloid- $\beta$  ELISA analysis of the older PDAPP mice was consistent with the immunohistochemical observations. In untreated PDAPP mice, the median level of total  $\text{A}\beta$  in the cortex at 12 months was 1,600 ng per g (wet weight); this had increased to 8,700 ng per g by 15 months<sup>12</sup>. At 18 months the value was 22,000 ng per g, an increase of more than 10-fold during the course of the experiment<sup>12</sup>. PBS-treated animals had comparable levels of total amyloid- $\beta$  at 15 months (8,600 ng per g), and 19,000 ng per g at 18 months. In contrast,  $\text{A}\beta_{42}$ -treated animals had 81% less total amyloid- $\beta$  at 15 months (1,600 ng per g) than the PBS-immunized group. Significantly less total amyloid- $\beta$  (5,200 ng per g) was found at 18 months when the  $\text{A}\beta_{42}$  and PBS groups were compared, representing a 72% reduction ( $P = 0.0001$ ) in the amyloid- $\beta$  that would have otherwise been present. Similar results were obtained when cortical levels of  $\text{A}\beta_{42}$  were compared, namely that the  $\text{A}\beta_{42}$ -treated group contained much less  $\text{A}\beta_{42}$ , and the differences between the  $\text{A}\beta_{42}$  and PBS groups were significant at 15 months ( $P = 0.04$ ) and 18 months ( $P = 0.0001$ ). In contrast, cortical levels of APP decreased by less than 10% of those in 12-month-old untreated animals (data not shown)<sup>12</sup>. These findings argue against the possibility that the reduction in amyloid- $\beta$  deposition seen in the treated mice is due to an alteration in APP metabolism.

The progression of neuritic pathology was significantly reduced in the frontal cortex of  $\text{A}\beta_{42}$ -treated compared with PBS-treated mice (Table 1). At 15 months of age, the neuritic plaque burden in



**Figure 5** Reduction of  $\text{A}\beta$  burden in the entorhinal and retrosplenial cortex of older PDAPP mice following  $\text{A}\beta_{42}$  injection.  $\text{A}\beta$  deposition in the retrosplenial (RSC) and entorhinal (EC) cortices of 15-month-old PBS- (a, b) and  $\text{A}\beta_{42}$ - (c, d) injected mice with  $\text{A}\beta$  burdens representative of the median values of their respective groups.  $\text{A}\beta$  deposition was greatly reduced in the RSC of  $\text{A}\beta_{42}$ -injected mice compared with the PBS group (compare a and c). No  $\text{A}\beta$  was detected in the EC of  $\text{A}\beta_{42}$ -injected mice (d), in contrast to the PBS group (b). Scale bar in d corresponds to all panels.



**Figure 6** Altered  $\text{A}\beta$  burden in the hippocampus of older  $\text{A}\beta_{42}$ -treated mice. Distribution of hippocampal  $\text{A}\beta$  in  $\text{A}\beta_{42}$ -injected brains (b), compared with the PBS group (a) at 18 months of age. In the PBS group, the characteristic appearance of diffuse (asterisks) and compacted (arrow)  $\text{A}\beta$  deposits was evident (a). This pattern was markedly altered in a number of  $\text{A}\beta_{42}$ -immunized mice (b), with the absence of diffuse deposits and an unusual punctate pattern of  $\text{A}\beta$  immunoreactivity associated with cells (b, arrow). The distribution of MHC II-labelled (Pharmingen) cells in a near adjacent section (c) corresponded to the pattern of  $\text{A}\beta$  positive cellular labelling shown in b. No such obvious cell  $\text{A}\beta$  staining was found in the PBS group (compare a and b). Scale bar in c corresponds to all panels.

**Table 1** Image analysis of neuritic plaque and astrocytic burden in  $A\beta_{42}$ -treated PDAPP mice

	15 months		18 months	
	PBS	$A\beta_{42}$ -treated	PBS	$A\beta_{42}$ -treated
Neuritic plaque (%)	0.32	0.02	0.49	0.22
Astrocytosis (%)	4.76	1.89	5.21	3.20

Quantitative image analysis of neuritic plaques and astrocytosis was performed using antibody 8E5 to human A $\beta$  and anti-GAP, respectively. Methods are described in Fig 1 legend. Reduction of neuritic plaques burden at ages 15 and 18 months by A $\beta_{42}$  treatment was statistically significant ( $P = 0.03$  and  $0.01$ , respectively), as was reduction of astrocytosis at the same time points ( $P = 0.01$  and  $0.03$ , respectively).

the  $A\beta_{42}$ -treated mice was reduced by 84% compared with the PBS group (0.05% and 0.32%,  $P = 0.03$ ). Reduction in the neuritic-plaque pathology was similarly maintained between the two groups at 18 months of age, where the degree of neuritic dystrophy was reduced by 55% in the  $A\beta_{42}$ -treated mice (0.22% and 0.49%;  $P = 0.01$ ).

Reactive astrocytosis was also significantly reduced in the retrosplenial cortex of  $A\beta_{42}$ -treated mice compared with PBS-treated mice at both 15 and 18 months of age. The per cent of astrocytosis in the PBS group increased between 15 and 18 months from 4.29% to 5.21%.  $A\beta_{42}$  treatment suppressed the development of astrocytosis at both time points to 1.89% and 3.2%, respectively. These differences represent a 56% reduction ( $P = 0.01$ ) at 15 months of age and a 34% decrease ( $P = 0.03$ ) at 18 months of age in the  $A\beta_{42}$ -treated group.

In summary, immunization with  $A\beta_{42}$  greatly reduced the development of the AD-like pathology that otherwise occurs in the PDAPP mouse. Immunization preceding plaque development profoundly affected the occurrence of new lesions, as the progression of  $\beta$ -amyloidosis and associated neuropathology was essentially wholly blocked, as seen both in the entire brain of the young animals and in at-risk brain regions of the older animals. Amyloid- $\beta$  immunization also significantly retarded the progression of existing pathology in affected regions of the older animals. Outcomes of A $\beta$ -plaque burden, neuritic dystrophy and gliosis were all significantly improved by  $A\beta_{42}$  treatment in both young and old animals. In addition, the mechanism resulting in plaque reduction did not seem to produce any obvious signs of damage to the neuropil of  $A\beta_{42}$ -immunized animals. Histological examination of several organs, including brain and kidney, revealed no signs of immune-mediated complications, despite the high levels of human A $\beta$  expressed in these tissues and the significant antibody titre to endogenous mouse A $\beta$  peptide (data not shown).

To our knowledge, this is the first report of a clinically relevant treatment that reduces the progression of AD-like neuropathology in a transgenic animal model of the disease. Although it remains unproven, it is not unreasonable to expect that a similar reduction of neuropathology in AD patients would be of clinical benefit. Although our understanding of the precise aspects of the immune response that result in reduced pathology is incomplete, we have shown that  $A\beta_{42}$  immunization results in the generation of anti-A $\beta$  antibodies and that A $\beta$ -immunoreactive monocytic/microglial cells appear in the regions of remaining plaques. Thus, one possible mechanism of action is that anti-A $\beta$  antibodies facilitate clearance of amyloid- $\beta$  either before deposition, or after plaque formation, by triggering monocytic/microglial cells to clear amyloid- $\beta$  using signals mediated by Fc receptors.

It has been suggested that a chronic inflammatory state exists in the brain of patients with Alzheimer's disease: specifically the levels of complement, cytokines and acute-phase proteins are raised<sup>14</sup>. These observations have led to the hypothesis that anti-inflammatory regimens might be of therapeutic value. The findings presented here argue that an alternative approach, one that augments a highly specific immune response, can markedly reduce pathology in an animal model of the disease. Collectively, the results suggest that

amyloid- $\beta$  immunization may prove beneficial for both the treatment and prevention of Alzheimer's disease. □

## Methods

**Immunization procedures.** A $\beta$  peptide was freshly prepared from lyophilized powder for each set of injections. For immunizations, 2 mg A $\beta_{42}$  (human A $\beta_{1-42}$ ; US Peptides) was added to 0.9 ml deionized water and the mixture was vortexed to generate a relatively uniform suspension. A 100- $\mu$ l aliquot of  $10 \times$  PBS (where  $1 \times$  PBS is 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5) was added. The suspension was vortexed again and incubated overnight at 37°C for use the next day. Serum amyloid-P component immunogens were prepared using mouse SAP amino acids 77–85 and 164–173, each conjugated to sheep anti-mouse IgG (Jackson Immunochemicals) as described<sup>15</sup>. A $\beta_{42}$  or SAP peptides (100  $\mu$ g antigen per injection) were emulsified 1:1 (v/v) with complete Freund's adjuvant for the first immunization, followed by a boost in incomplete Freund's adjuvant at 2 weeks, and monthly thereafter. A $\beta_{42}$  or SAP in PBS alone was injected from the fifth immunization onward. Titres were determined by serial dilutions of sera against either aggregated A $\beta_{42}$  or SAP protein which had been coated onto microtitre wells. Detection used goat anti-mouse immunoglobulin conjugated to horseradish peroxidase and slow-TMB (3,3',5,5'-tetramethyl benzidine; Pierce) substrate. Titres were defined as the dilution yielding 50% of the maximal signal.

**Neuropathology quantification.** To quantify amyloid burden, PDAPP mouse brain tissue was fixed in 4% paraformaldehyde, cut to 40- $\mu$ m coronal sections and reacted with an anti-A $\beta$  biotinylated antibody, 3D6, as described previously<sup>16</sup>. Quantitative image analysis was performed using a Videometrics 150 Image Analysis System (Oncor) linked to a Nikon Microphot-FX microscope through a CCD video camera. The image of the immunoreacted section was stored in a video buffer and a specific brain region (hippocampus or cortex) was manually outlined and the total pixel area occupied by the structure determined. A monochromatic-based threshold was set to select pixels corresponding to immunolabelled structures. The per cent of the brain region occupied by the labelled pixels was then calculated. For all image analyses, six sections at the level of the dorsal hippocampus, each separated by consecutive 240- $\mu$ m intervals, were evaluated for each animal. In all cases, the treatment status of the animals was unknown to the observer. Mann-Whitney nonparametric analysis was performed using Statview software (SAS Institute, Cary, NC). Similar methodologies were used to quantify neuritic dystrophy and gliosis. Specific regions are indicated in the appropriate figures.

Received 6 April; accepted 5 May 1999

- Hyman, B. T. New neuropathological criteria for Alzheimer disease. *Arch Neurol* **55**, 1174–1176 (1998).
- Tanzi, R. E. *et al.* The gene defects responsible for familial Alzheimer's disease. *Neurobiol Dis* **3**, 159–168 (1996).
- Hardy, J. New insights into the genetics of Alzheimer's disease. *Ann Med* **28**, 255–258 (1996).
- Cutler, M. *et al.* Mutation of the  $\beta$ -amyloid precursor protein in familial Alzheimer's disease increases  $\beta$  protein production. *Nature* **360**, 672–674 (1992).
- Schmechel, D. *et al.* Secreted amyloid  $\beta$  protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 APP mutations linked to familial Alzheimer's disease. *Nature Med* **2**, 861–870 (1996).
- Suzuki, N. *et al.* An increased percentage of long amyloid  $\beta$  protein secreted by familial amyloid  $\beta$  protein (A $\beta$ APP717) mutants. *Science* **264**, 1330–1340 (1994).
- Cutler, M. *et al.* Mutant presenilins of Alzheimer's disease increase production of  $\beta$ -residue amyloid- $\beta$  protein in both transfected cells and transgenic mice. *Nature Med* **3**, 67–72 (1997).
- Borchelt, D. R. *et al.* Familial Alzheimer's disease-linked presenilin 1 variants exhibit A $\beta$ 1-42:1-40 ratio *in vitro* and *in vivo*. *Neuron* **17**, 1005–1013 (1996).
- Benito, J. *et al.* Visualization of A $\beta$ 43 and A $\beta$ 40 in senile plaques with anti- $\beta$ -amyloid A $\beta$  monoclonals: evidence that an initially deposited species is A $\beta$ 42(43). *Neuron* **13**, 65–73 (1994).
- Lippa, C. F., Neill, L. E., Mori, H. & St George-Hyslop, P. A $\beta$  42 deposition precedes other changes in PS-1 Alzheimer's disease. *Neuron* **25**, 1117–1118 (1998).
- Garzon, M. *et al.* Mutant amyloid type neuropathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein. *Nature* **373**, 523–527 (1995).
- Johnson, W. *et al.* Amyloid precursor protein processing and A $\beta$ 42 deposition in a transgenic mouse model of Alzheimer disease. *Proc Natl Acad Sci USA* **94**, 1550–1555 (1997).
- Luong, S. S. *et al.* Evidence for loss of production of an acute phase response apolipoprotein serum amyloid A in Alzheimer's disease brain. *Neurosci Lett* **225**, 7–10 (1997).
- McGeer, E. L. & McGeer, P. L. The role of the immune system in neurodegenerative disorders. *Mov Disord* **12**, 855–858 (1997).
- Seubert, P. *et al.* Isolation and quantification of soluble Alzheimer's  $\beta$ -peptide from biological fluids. *Nature* **359**, 325–327 (1993).
- Seubert, P. *et al.* Secretion of  $\beta$ -amyloid precursor protein cleaved at the amino terminus of the  $\beta$ -amyloid peptide. *Nature* **361**, 260–263 (1993).

**Acknowledgements.** We thank Ray Burke for helpful comments.

Correspondence and requests for materials should be addressed to D.S. (e-mail: dschm@calypso.com).

# Peripherally administered antibodies against amyloid $\beta$ -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease

FRÉDÉRIQUE BARD, CATHERINE CANNON, ROBIN BARBOUR, RAE-LYN BURKE, DORA GAMES, HENRY GRAJEDA, TERESA GUIDO, KANG HU, JIPING HUANG, KELLY JOHNSON-WOOD, KAREN KHAN, DORA KHOLODENKO, MIKE LEE, IVAN LIEBERBURG, RUTH MOTTER, MINH NGUYEN, FÉRDIE SORIANO, NICKI VASQUEZ, KIM WEISS, BRENT WELCH, PETER SEUBERT, DALE SCHENK & TED YEDNOCK

Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, California 94080, USA

Correspondence should be addressed to F.B.; email: [fbard@elanpharma.com](mailto:fbard@elanpharma.com)

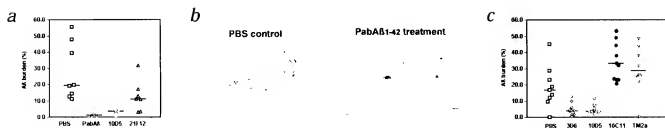
One hallmark of Alzheimer disease is the accumulation of amyloid  $\beta$ -peptide in the brain and its deposition as plaques. Mice transgenic for an amyloid  $\beta$  precursor protein (APP) mini-gene driven by a platelet-derived (PD) growth factor promoter (PDAPP mice), which overexpress one of the disease-linked mutant forms of the human amyloid precursor protein, show many of the pathological features of Alzheimer disease, including extensive deposition of extracellular amyloid plaques, astrogliosis and neuritic dystrophy<sup>1,2</sup>. Active immunization of PDAPP mice with human amyloid  $\beta$ -peptide reduces plaque burden and its associated pathologies<sup>3</sup>. Several hypotheses have been proposed regarding the mechanism of this response<sup>4,5</sup>. Here we report that peripheral administration of antibodies against amyloid  $\beta$ -peptide, was sufficient to reduce amyloid burden. Despite their relatively modest serum levels, the passively administered antibodies were able to enter the central nervous system, decorate plaques and induce clearance of preexisting amyloid. When examined in an *ex vivo* assay with sections of PDAPP or Alzheimer disease brain tissue, antibodies against amyloid  $\beta$ -peptide triggered microglial cells to clear plaques through Fc receptor-mediated phagocytosis and subsequent peptide degradation. These results indicate that antibodies can cross the blood-brain barrier to act directly in the central nervous system and should be considered as a therapeutic approach for the treatment of Alzheimer disease and other neurological disorders.

We administered antibodies against amyloid  $\beta$ -peptide (A $\beta$ ) by intraperitoneal injection to mice transgenic for an A $\beta$  precursor protein (APP) mini-gene driven by a platelet-derived (PD) growth factor promoter (PDAPP mice). In the first experiment, 8- to 10-month-old heterozygous PDAPP mice ( $n = 8$ ) received either PBS, one of two different mouse monoclonal antibodies against A $\beta$  (10D5 or 21F12) or a polyclonal immunoglobulin (Ig) fraction obtained from mice immunized with the 42-amino acid form of A $\beta$  (pAbA $\beta_{42}$ ). The mice received weekly injections of antibody for six months, maintaining a constant antibody serum concentration throughout the course of the experiment. We used quantitative image analysis to determine amyloid burden and enzyme linked immunosorbent assay (ELISA) to determine A $\beta_{42}$  levels in the cortex, as described<sup>6</sup>. Relative to control treated mice, the polyclonal immunoglobulin fraction against A $\beta$  and one of the monoclonal antibodies (10D5) re-

duced plaque burden by 93% and 81%, respectively (Fig. 1a,  $P < 0.005$ ). There were similar reductions in cortical levels of A $\beta_{42}$  by ELISA measurements (6200, 4890 and 13800 ng/g tissue for 10D5, pAbA $\beta_{42}$  and PBS, respectively). Although 21F12 seemed to have a relatively modest effect on plaque burden, there was no substantial reduction as determined by ELISA measurements (13,580 ng/g). The effect of pAbA $\beta_{42}$  on plaque burden was demonstrated in micrographs of brain sections obtained from mice having the median level of plaque burden within their respective groups. Most of the diffuse deposits and many of the larger compacted plaques were absent in mice treated with pAbA $\beta_{42}$  compared to those in the control groups.

In a second study, we repeated 10D5 treatment and tested two additional antibodies against A $\beta$ , 3D6 and 16C11 (Fig. 1c). Control groups received either PBS or an irrelevant isotype-matched antibody (TM2a). The experimental design was the same as in the previous study except for the age of the mice (11.5–12 months old). Once again, after 6 months of treatment, 10D5 reduced plaque burden by greater than 80% compared with that of either the PBS or isotype-matched antibody controls ( $P = 0.003$ ). Treatment with 3D6 was equally effective, producing an 86% reduction in plaque burden ( $P = 0.003$ ). In contrast, 16C11 failed to have any effect on plaque burden. Unlike actively immunized mice, mice receiving exogenous antibodies against A $\beta$  did not demonstrate a T-cell proliferative response to A $\beta$  when their splenocytes were examined *in vitro*, indicating that a T-cell response is not required for amyloid plaque clearance (data not shown). These results indicate that in the absence of T-cell immunity, antibodies against A $\beta$  peptide are sufficient to decrease amyloid deposition in PDAPP mice.

To determine whether the peripherally administered antibodies against A $\beta$  had entered the central nervous system (CNS) to act directly on plaques, we examined brain sections taken from saline-perfused mice at the end of the second study. We exposed unfixed cryostat brain sections to a fluorochrome tagged reagent against mouse immunoglobulin. Plaques within the brains of the 10D5- and 3D6-treated groups were strongly 'decorated' with antibody, whereas there was no staining in the 16C11-treated group (Fig. 2a, top). To show the full extent of plaque deposition, we immunostained serial sections of each brain with an exogenous antibody against A $\beta$ , followed by the secondary reagent (Fig. 2a, bottom). After peripheral administration, 10D5 and 3D6 had gained access to most plaques within the CNS where they



**Fig. 1** A $\beta$  burden in the frontal cortex of PDAPP mice is reduced after 6 months of treatment with antibodies against A $\beta$ . **a** and **c**, Percentage of frontal cortex area occupied by A $\beta$  deposits shown in individual mice sorted

by treatment group ( $n = 8$ ). Horizontal lines indicate median values. **b**, Brain sections of the cortex and hippocampus obtained from mice with the median level of plaque burden in their respective groups. Scale bar represents 250  $\mu$ m.

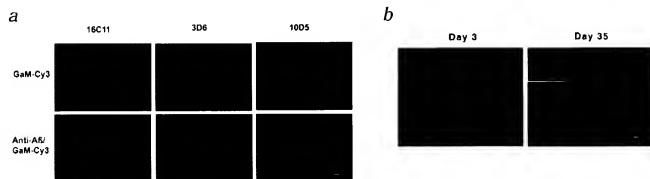
may have directly triggered amyloid clearance. It is likely that 16C11 also had access to the plaques but was unable to bind (described below).

We undertook a separate study to determine if antibody treatment resulted in the clearance of preexisting amyloid or simply prevented formation of new plaques. We administered 3D6 or control antibody to 13-month-old heterozygous PDAPP mice, then examined the brains for total plaque burden after 3 and 35 days of treatment. Although there was no obvious change in the number of large plaques over the short treatment period (Fig. 2b), there seemed to be a reduction in diffuse amyloid and small aggregates of A $\beta$  (Fig. 2b, inset). By image analysis of the frontal cortex, nearly 60% of the small plaques and diffuse amyloid, corresponding to pixels with intermediate to low intensity, had been eliminated during the intervening 32 days of treatment (Fig. 2c;  $P = 0.001$ ). These results confirm that the antibody treatment triggered clearance of preexisting amyloid.

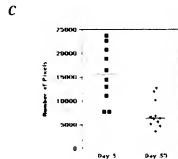
To further examine the effect of antibodies on plaque clearance, we established an *ex vivo* assay in which primary microglial cells were cultured with unfixed cryostat sections of either

PDAPP mouse or human Alzheimer disease (AD) brains. After 24 hours, we fixed, permeabilized and immunostained the cultures to follow the fate of amyloid  $\beta$ -peptide, and visualized the exogenous microglial cells with a nuclear stain. In PDAPP brain sections assayed in the presence of 16C11 (one of the antibodies against A $\beta$  that was not efficacious *in vivo*), amyloid  $\beta$ -protein plaques remained intact and there was no phagocytosis. In contrast, after culture of adjacent sections in the presence of 10D5, amyloid deposits were mostly eliminated and the microglial cells showed many phagocytic vesicles containing A $\beta$  (Fig. 3a, top). We obtained identical results with AD brain sections: 10D5 induced phagocytosis of AD plaques, whereas 16C11 was inactive (Fig. 3a, bottom). Furthermore, the assay was equally effective with either mouse or human microglial cells, and with mouse, rabbit or primate antibodies against A $\beta$  (data not shown).

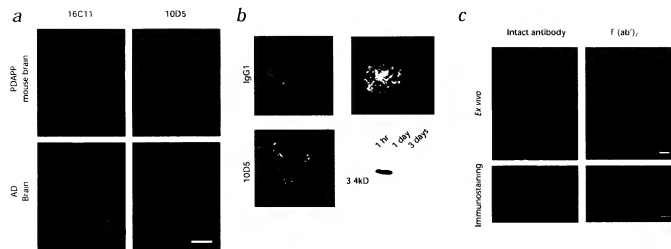
By comparison of six different antibodies tested in both systems, the *ex vivo* assay was predictive of *in vivo* efficacy (Table 1). Antibodies 10D5, 3D6 and pabA $\beta_{1-42}$  were all active in the *ex vivo* assay and demonstrated efficacy *in vivo*. In contrast, 16C11, 2F12 and the control antibody TM2a were inactive in



**Fig. 2** After peripheral administration, 10D5 and 3D6 enter the CNS, bind to A $\beta$  plaques and trigger amyloid clearance. **a**, Cryostat sections of brains from treated mice were exposed directly to fluorescently conjugated goat antibody against mouse immunoglobulin (top). Serial sections from the same brains were exposed to 10D5 before GaM-Cy3 reagent to show full extent of plaque burden (bottom). Scale bar represents 100  $\mu$ m. Anti-A $\beta$ , antibody against A $\beta$ . **b**, Total plaque burden in the hippocampus and cortex in mice treated for 3 and 35 d with 3D6. The plaque burden is greatly reduced by 3D6 and 10D5 compared with that of 16C11. Scale bar represents 120  $\mu$ m. Insets, high-powered magnification of individual large plaques within the cortex of the corresponding section (scale bar represents 20  $\mu$ m). There is a lack of amyloid fibrils and small aggregates surrounding the plaque in the day-35 section. **c**, Quantification of diffuse amyloid and small plaques shows a 60% reduction between the 3- and 35-day treatment groups ( $n = 10$ /group;  $P = 0.001$ ).



## ARTICLES



**Fig. 3** Fc receptor-mediated phagocytosis of A $\beta$  in an *ex vivo* assay. **a**, Mouse microglia cultured with unfixed cryostat sections of PDAPP (top) or AD (bottom) brain in the presence of antibodies against A $\beta$ . In the presence of 10D5, A $\beta$  (red) localizes to vesicles within the membrane boundaries of microglial cells; in the presence of 16C11, plaques remain intact and there is no evidence of cellular staining. Scale bar represents 10  $\mu$ m. **b**, Confocal microscopy shows that in the presence of control IgG1 (top), microglial cells (red) are in a confocal plane above the tissue section and A $\beta$  (green) remain in plaques within the tissue plane (differ-

ent planes of the same field are shown here). In the presence of 10D5 (bottom left), nearly all A $\beta$  is localized within the microglial cells. Bottom right, western blot analysis with a polyclonal antibody against A $\beta$  shows complete degradation of A $\beta$  within 3 d (left margin, molecular size marker). **c**, Fc receptor-mediated phagocytosis of A $\beta$ , shown by *ex vivo* assay in the presence of intact or F(ab')<sub>2</sub> fragments of 3D6 (top). Scale bar represents 50  $\mu$ m. A $\beta$  plaques in consecutive sections are equally 'decorated' with intact 3D6 and its F(ab')<sub>2</sub> fragments (bottom). Scale bar represents 300  $\mu$ m.

both assays. Comparison of other antibody characteristics showed that whereas 16C11 and 21F12 bound to aggregated synthetic A $\beta$  peptide with high avidity, they were unable to bind to plaques in unfixed brain sections. This result is consistent with the inability of these two antibodies to decorate plaques after *in vivo* administration (as shown for 16C11 in Fig. 2a, top) and explains their inability to trigger plaque clearance. We found no correlation between antibody efficacy and affinity for soluble A $\beta$  3D6 and the inactive antibodies captured soluble A $\beta$  with moderate to high affinity, whereas 10D5 could not capture the soluble peptide. These results indicate that recognition and clearance of deposited A $\beta$  is an important component of antibody efficacy *in vivo* and may be more important than a mechanism involving the prevention of plaque deposition by recognition of soluble A $\beta$ .

We then used confocal microscopy to confirm that A $\beta$  was internalized during the course of the *ex vivo* assay (Fig. 3b). In the presence of control antibody, the exogenous microglial cells (red) remained in a confocal plane above the tissue section and contained no phagocytic vesicles, whereas A $\beta$  (green) remained

in plaques within the tissue plane (Fig. 3b, top). In the presence of 10D5, nearly all plaque amyloid was contained in vesicles within the exogenous microglial cells (Fig. 3b, bottom). This result is consistent with the earlier finding of intracellular A $\beta$  immunoreactivity within macrophage and microglial cells in the CNS of immunized mice<sup>4</sup>. To determine the fate of the internalized peptide, we assessed 10D5-treated cultures by western blot analysis (Fig. 3b, bottom right). At 1 hour, when no phagocytosis had yet occurred, reaction with a polyclonal antibody against A $\beta$  showed a strong 4 kDa band, corresponding to A $\beta$  peptide. A $\beta$  immunoreactivity decreased at day 1 and was absent by day 3. There was no degradation of A $\beta$  staining in cultures with control IgG1. Thus, in contrast to what has been reported for A $\beta$  with other microglial scavenging pathways<sup>5,7</sup>, antibody-mediated phagocytosis of A $\beta$  led to its degradation.

To determine if phagocytosis in the *ex vivo* assay was mediated by Fc, we prepared F(ab')<sub>2</sub> fragments of 3D6. Although the antibody fragments retained their full ability to react with plaques (Fig. 3c, bottom), they were unable to trigger microglial cell phagocytosis (Fig. 3c, top). In addition, phagocytosis induced by

the whole 3D6 antibody was inhibited by antibodies specific for Fc receptors on either human or mouse microglial cells (data not shown). These results indicate that *in vivo* clearance of A $\beta$  occurred through Fc receptor-mediated phagocytosis.

In summary, we have shown that passively administered antibodies against A $\beta$  peptide reduced the extent of plaque deposition in a mouse model of Alzheimer dis-

**Table 1** The *ex vivo* assay as predictor of *in vivo* efficacy.

Antibody	Isotype	Avidity for aggregated A $\beta$ (pM)	Affinity for soluble A $\beta$ (nM)	Binding to A $\beta$ plaques	<i>Ex vivo</i> efficacy	<i>In vivo</i> efficacy
monoclonal						
3D6	IgG2b	470	< 30	+	+	+
10D5	IgG1	43	no capture	+	+	+
16C11	IgG1	90	110	—	—	—
21F12	IgG2a	500	80	—	—	—
TM24	IgG1	—	—	—	—	—
PabA $\beta$ <sub>1-42</sub>	mix	600	nd	+	+	+

The affinity of 10D5 was too low to capture soluble A $\beta$  and could not be measured in this assay. nd, not done.

ease. Antibody entry into the CNS was not due to abnormal leakage of the blood-brain barrier, as there was no increase in vascular permeability in PDAPP mice. In addition, the concentration of endogenous immunoglobulins in the brain parenchyma of aged PDAPP mice was the same as in nontransgenic mice, representing 0.1% of the antibody concentration in serum (regardless of isotype; data not shown). Similar findings have been published for antibody levels in human cerebrospinal fluid. These studies indicate that monoclonal antibodies are able to enter the CNS at therapeutically relevant levels, and that antibodies may be considered not only for the treatment of Alzheimer disease, but possibly for other CNS disorders as well.

## Methods

**Immunization procedures.** Monoclonal antibodies against A $\beta$  were raised against synthetic peptide fragments derived from different regions of A $\beta$ , and coupled to a carrier protein as described<sup>19</sup>. Polyclonal immune sera were pooled from 100 mice that had been immunized with full length A $\beta$ , and the immunoglobulin fraction was isolated by standard ammonium sulfate precipitation. After purification, all antibodies were dialyzed against PBS and had a final endotoxin level of < 1 endotoxin unit (EU), as measured by the limulus amoebocyte gel clot assay (Associates of Cape Cod, Cape Cod, Massachusetts). Antibody titers in serum were determined weekly as described<sup>1</sup>.

**Ex vivo assay.** Cryostat sections (10  $\mu$ m in thickness) of PDAPP mouse or human AD brains (postmortem interval, less than 3 h) were 'thaw-mounted' onto polylysine-coated, round glass coverslips and placed in wells of 24-well tissue culture plates. The coverslips were washed twice with assay medium consisting of hybridoma-serum free medium (H-SFM; Life Technologies) plus 1% FBS, glutamine, penicillin/streptomycin, and 5 ng/ml recombinant mouse GM-CSF granulocyte-macrophage colony-stimulating factor (R&D Systems, Minneapolis, Minnesota). Antibodies (control or against A $\beta$ ) were added at a 2X concentration (5  $\mu$ g/ml final) for 1 h. Microglial cells were then seeded at a density of  $0.8 \times 10^4$  cells/ml assay medium. In some experiments, medium contained 10  $\mu$ g/ml soybean trypsin inhibitor (Life Technologies). The cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO<sub>2</sub> for 24 h or longer. After incubation, cultures were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X100. Sections were stained with biotinylated 3D6, followed by streptavidin/Cy3 conjugate (Jackson ImmunoResearch, West Grove, Pennsylvania). Cultures were observed with an inverted fluorescent microscope (TE300; Nikon, Melville, New York) and photomicrographs were taken with a SPOT digital camera using SPOT software

(Diagnostic Instruments, Sterling Heights, Michigan). For western blot analysis, cultures were extracted with 8 M urea, diluted 1:1 in reducing tricine sample buffer, and loaded onto a 16% tricine gel (Novex, San Diego, California). After transfer onto Immobilon, blots were exposed to 5  $\mu$ g/ml pAbA $\beta$ , followed by horseradish peroxidase-conjugated antibody against mouse, and were developed with an ECL kit (Amersham).

**Microglia culture.** Microglial cells were obtained from cerebral cortices of neonate DBA/2N mice (1–3 days old). Cortices were mechanically dissociated in Hanks' balanced salt solution with 50  $\mu$ g/ml DNase I (both from Sigma). Dissociated cells were filtered through a 100  $\mu$ m cell strainer (Falcon, Heidelberg, Germany) and centrifuged at 200g for 5 min. Pellets were resuspended in growth medium (high-glucose DMEM, 10% FBS and 25 ng/ml recombinant mouse granulocyte-macrophage colony-stimulating factor), and cells were plated at a density of two brains per T-75 plastic culture flask. After 7–9 d, the flasks were rotated at 200 rpm using a Lab-Line orbital shaker with a 19-mm orbit for 2 h at 37 °C. Cell suspensions were centrifuged at 200g and resuspended in assay medium.

## Acknowledgments

We thank K. Hoenow for technical support

RECEIVED 5 JUNE; ACCEPTED 20 JUNE 2000

- Games, D. et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein. *Nature* 373, 523–527 (1995).
- Masliah, E. et al. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein and Alzheimer's disease. *J. Neurosci.* 16, 5795–5811 (1996).
- Schenk, D. et al. Immunization with amyloid- $\beta$  attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400, 173–177 (1999).
- St. George-Hyslop, P. H. & Westaway, D. A. Alzheimer's disease: antibody clears senile plaques. *Nature* 400, 116–117 (1999).
- Duff, K. Curing amyloidosis: will it work in humans? *Trends Neurosci.* 22, 485–486 (1999).
- Paresse, D. M., Chung, H. & Maxfield, F. R. Slow degradation of aggregates of the Alzheimer's disease amyloid  $\beta$ -protein by microglial cells. *J. Biol. Chem.* 272, 29390–29397 (1997).
- Chung, H., Brazil, M. L., Sze, T. T. & Maxfield, F. R. Uptake, degradation, and release of fibrillar and soluble forms of Alzheimer's amyloid  $\beta$ -peptide by microglial cells. *J. Biol. Chem.* 274, 32301–32308 (1999).
- Thompson, E. J. & Keir, G. Laboratory investigation of cerebrospinal fluid proteins. *Ann. Clin. Biochem.* 27, 425–435 (1990).
- Johnson-Wood K. et al. Amyloid precursor protein processing and A $\beta$ 42 deposition in a transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 94, 1550–1555 (1997).
- Hyman, B. T. et al. Kunitz protease inhibitor-containing amyloid beta protein precursor immunoreactivity in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* 51, 76–83 (1992).